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Measurement of nutrient availability in feedstuffs for Florida pompano and development of formulated diets for pompano aquaculture

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MEASUREMENT OF NUTRIENT AVAILABILITY IN FEEDSTUFFS FOR FLORIDA POMPANO AND DEVELOPMENT OF FORMULATED DIETS FOR POMPANO AQUACULTURE

A Thesis

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by

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My fish,
why fish,
do you die fish?

Why do you pass away,
while you’re still young and gay?

Wouldn’t you rather,
live a little longer;
so that when you die fish,
you can be on my dish?

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ABSTRACT

Consistently high market demand combined with low commercial catches have made Florida pompano *Trachinotus carolinus* one of the highest valued finfish from the Gulf of Mexico. Economic incentive has spurred interest in pompano culture since the 1950s, yet the body of nutritional research on pompano is still sparse. This study was conducted to measure the apparent digestibility of nutrients among a selection of common feed ingredients, and determine the optimum dietary protein level for juvenile Florida pompano at a fixed protein-to-energy ratio, in order to formulate nutritionally complete, cost-effective diets.

The four ingredients tested in the digestibility trial were soybean meal (SM), meat and bone meal (MB), fish meal (FM), and corn grain (CG). Apparent energy digestibility (AED) was similar for SM (67.4 ± 0.8 %) and MB (65.7 ± 8.2 %), while FM was significantly higher (105.1 ± 5.4 %), and CG was significantly lower (41.4 ± 0.5 %). Apparent crude protein digestibility (ACPD) varied by ingredient: SM (84.3 ± 0.5 %), MB (62.4 ± 6.1 %), FM (95.3 ± 1.9 %), and CG (71.4 ± 1.0 %). The apparent amino acid availability (AAAA) ranges mirrored the ACPD values: SM (78.4 ± 2.2 – 96.5 ± 1.0 %), MB (48.1 ± 9.5 – 84.7 ± 3.8 %), FM (89.0 ± 7.9 – 109.1 ± 14.6 %), and CG (40.0 ± 3.9 – 85.0 ± 0.4 %). In addition to AED and ACPD, AAAA values provide a previously unreported measure of the growth-promoting value of each ingredient for Florida pompano.

The second experiment was a growth trial that examined the growth effects of six dietary protein levels (36% - 56% CP) at a fixed digestible energy-to-protein ratio (9 kcal DE/g CP). Survival was poor throughout the experiment, which led to termination of the 36% CP treatment by week six. Fish growth performance during the remaining five treatments produced significantly higher weight gain for the 48% and 56% CP treatments than the 40% CP treatment. Broken-line regression indicated an ideal protein level of about 46% CP for Florida pompano diets containing 9 kcal DE/g CP.

CHAPTER 1

INTRODUCTION

Background

Florida pompano (*Trachinotus carolinus*), hereafter referred to as “pompano,” are sub-tropical members of the jack family (Carangidae) native to the western Atlantic Ocean (Gilbert 2002). Florida pompano are the most sought after member of the family; historically praised as a superior food and sport fish (Henshall 1884, Jordan and Everman 1905, Bardach et al. 1972, Hoese and Moore 1998). A coastal species, pompano range from Massachusetts to Brazil, including the Gulf of Mexico and the Caribbean coasts of Central and South America (Jordan and Evermann 1896). They are especially common along the Florida coasts (Fields 1962, Gilbert 1986, Watanabe 1995), where the majority of biological, ecological, and aquacultural research on pompano has been done (Berry and Iverson 1967; Moe et al. 1968; Finucane 1969; 1970a,b; Smith 1973; Armitage and Alevizon 1980; Gilbert 1986; Murphy et al. 1996; Heilman and Spieler 1999; Nelson and Murphy 2001; Muller et al. 2002). High demand, good market price, and rapid growth catapulted pompano to the forefront of early marine aquaculture (mariculture) research (Berry and Iverson 1967, Moe et al. 1968, Finucane 1969, 1970a,b). Additional research on pompano culture has been done in Louisiana (Allen and Avault 1970, Weirich et al. 2006), Alabama (Swingle and Tatum 1971, Swingle 1972, Tatum 1972, Tatum and Trimble 1978, Trimble 1980, Williams et al. 1985), Texas (Holt and Strawn 1977; Rossberg and Strawn 1980a,b; Lazo et al. 1998), and Venezuela (Gomez and Scelzo 1982).

There are a total of 20 species in the genus *Trachinotus*. Two species overlap the Florida pompano’s native range (Fields 1962, Finucane 1969, Bardach et al. 1972, Gilbert 1986): permit (*T. falcatus*), a larger, more ventrally robust fish than the Florida pompano, and palometa (*T. goodei*). Both species have been cultured (Jory et al. 1985, Tucker and Jory 1991, Cole et al. 1997) and there has been interest in hybridization of pompano and permit (Jory et al. 1985, Watanabe 1995); although, no work in this area has been done. Currently there are experimental and commercial operations for the culture of snub-nosed pompano (*T. blochii*) and derbio (*T. ovatus*) in China, Croatia, Singapore, and Taiwan (Liao et al. 1995, Chou et al. 1995, Chou and Lee 1997, Tutman et al. 2004). Research on these closely-related species may contribute information beneficial to the culture of Florida pompano.

Biology

Pompano are relatively small (maximum size 3.6 kg; Gilbert 2002), laterally-compressed, silvery, schooling fish (Figure 1.1). They possess small sub-terminal mouths, which are used to comb through sand for their benthic prey (i.e., mollusks and crustaceans). Short, widely spaced gill rakers allow food to be separated from sand and detritus, while their pharyngeal teeth crush shells and other hard materials (Figure 1.2). A short esophagus directs food to a distensible y-shaped stomach (Figure 1.3). At the pyloric end of the stomach, the gall bladder inserts bile salts and enzymes into the gastrointestinal (GI) tract via a duct (Figure 1.4). The GI tract branches at this point between pyloric ceca and intestine (Figure 1.4). Florida pompano typically have 11 ceca (personal observation, n = 20), which are blind sacs that presumably aid in digestion and absorption by increasing intestinal surface area. Buddington and Diamond (1987) found that the pyloric ceca of trout fill and empty with small food particles (less than 150 microns in diameter), a process that may be similar in pompano. In this case, larger food particles probably enter directly into the intestine, which is relatively short in pompano (Figure 1.5). The ratio of intestine length (measured from the distal end of the stomach to the anus) to body length (I/B) in pompano is 0.80 ± 0.07 (personal observation; n = 10, 13.5 - 30 cm TL), which falls within the range presented by Bond (1996) for piscivorous species. The short gut evacuates feces in three hours (Williams et al. 1985); however, personal observations indicated fecal output continues until six hours post-feeding. The pompano swim bladder is reduced (Figure 1.6) and fish typically swim constantly, which is energetically costly. Because of the short gut transit time and active nature, diets formulated for pompano should contain readily available nutrients and energy in feedstuffs that can be digested easily.

FIGURE 1.1. Florida pompano, *Trachinotus carolinus*, profile illustrating lateral compression, narrow caudal peduncle, high aspect ratio caudal fin, ovate shape, silvery coloration, and small sub-terminal mouth.

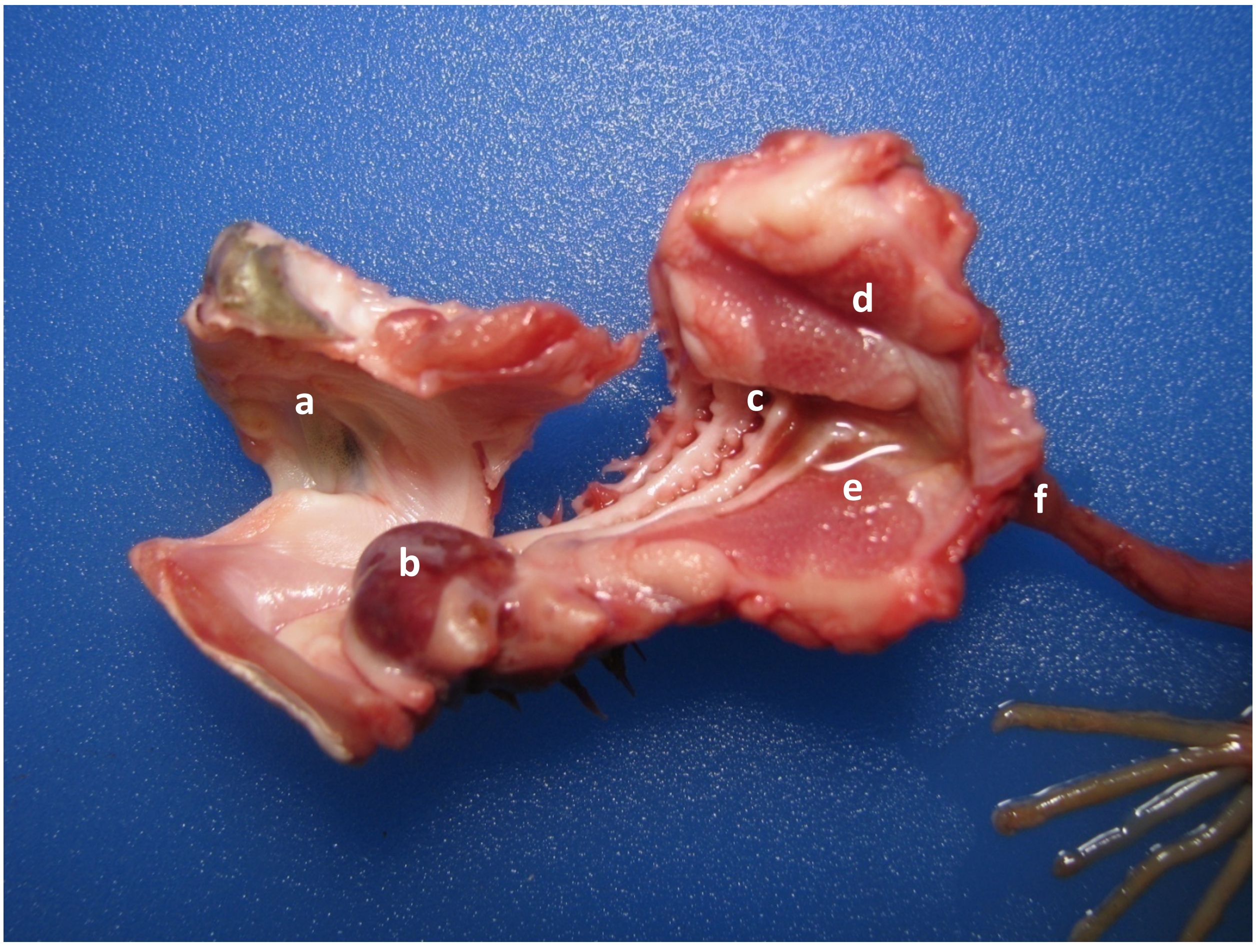

FIGURE 1.2. Removed buccal cavity of Florida pompano. (a) mouth, (b) tongue (swollen from goiter), (c) gill rakers, (d) upper pharyngeal teeth, (e) lower pharyngeal teeth, and (f) esophagus.

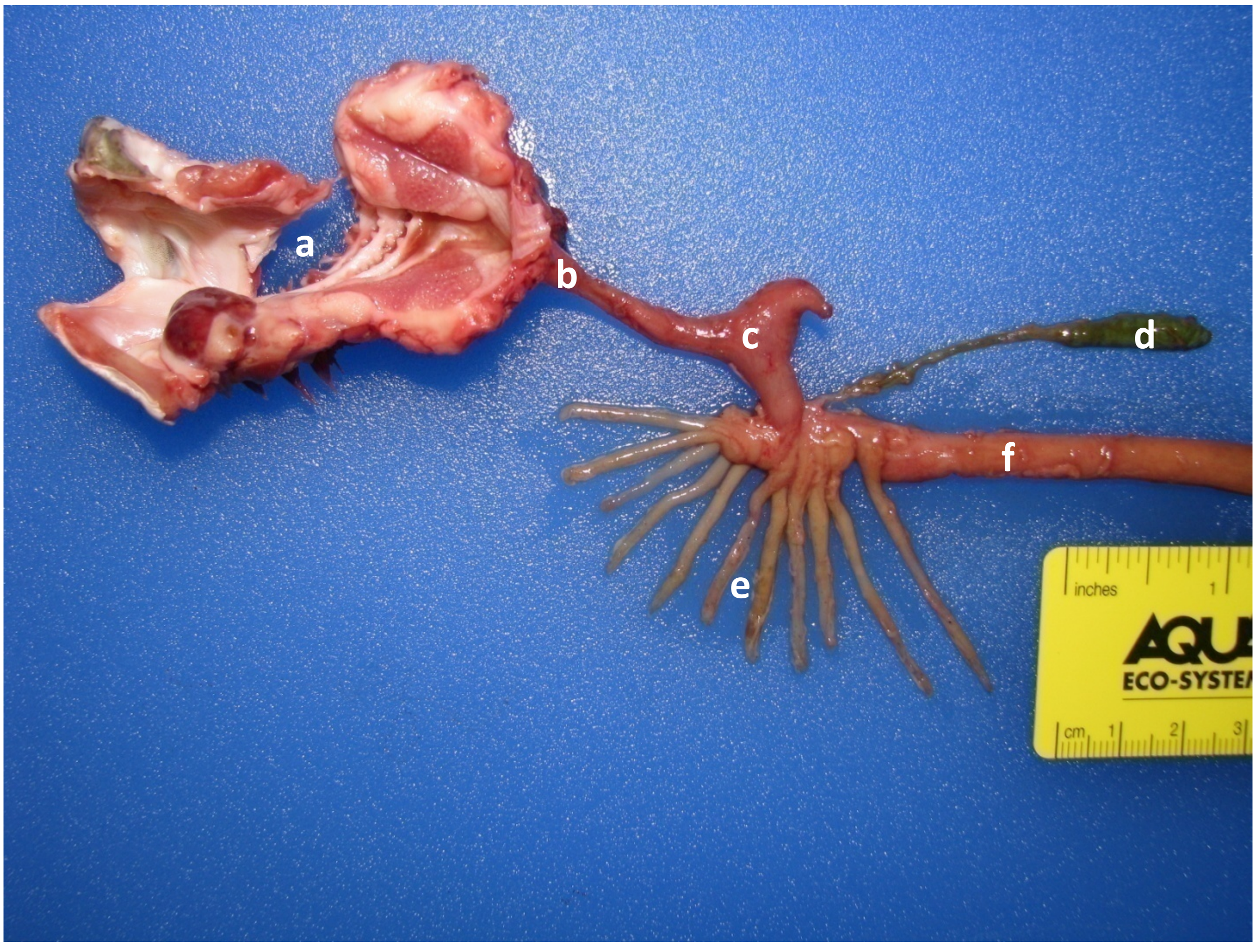

Figure 1.3. Removed GI tract from a Florida pompano. (a) buccal cavity, (b) esophagus, (c) y-shaped stomach, (d) gall bladder, (e) pyloric ceca, and (f) intestine.

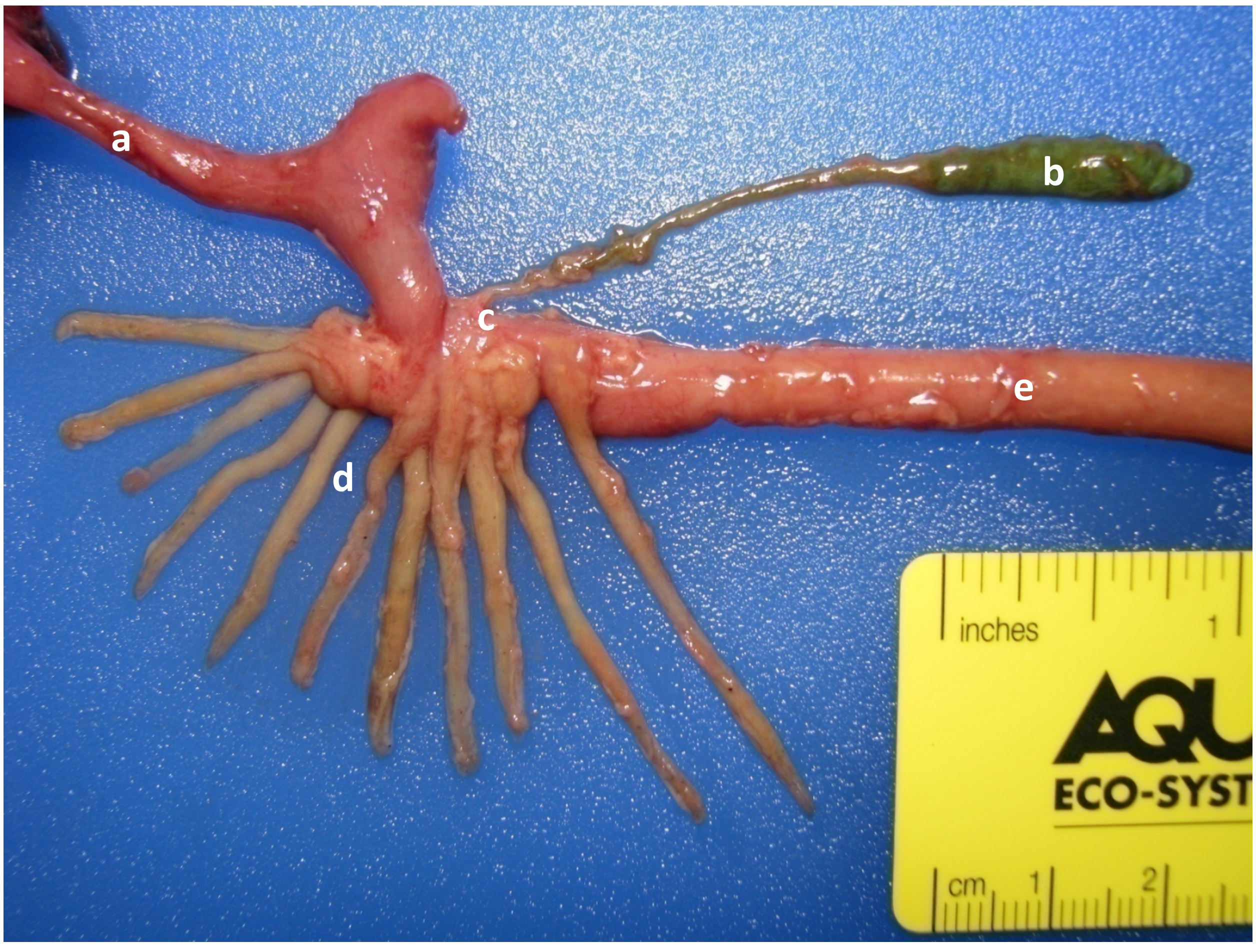

FIGURE 1.4. Close-up of removed GI tract from a Florida pompano. (a) esophagus, (b) gall bladder, (c) gall bladder insertion into intestine, (d) pyloric ceca, and (e) intestine.

FIGURE 1.5. Removed GI tract of a sub-adult Florida pompano illustrating intestinal length in relation to body length. The ratio of intestine length to body length (I/B) in pompano is 0.80 ± 0.07 (n = 10) for fish ranging from 13.5 - 30 cm TL, a value typical of piscivorous fish.

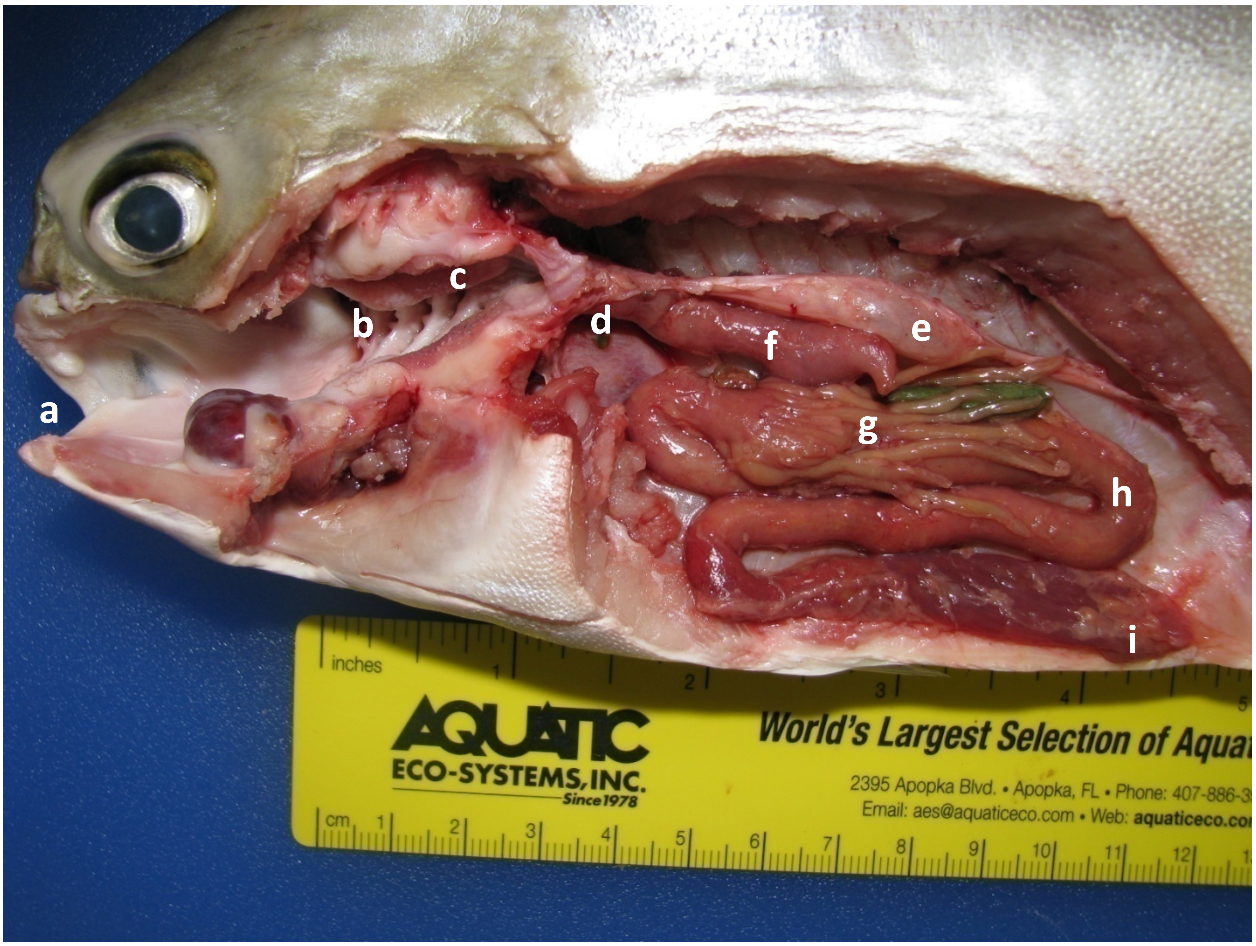

FIGURE 1.6. Dissection of the anterior portion of a sub-adult Florida pompano, with some organs removed, illustrating the general orientation of the GI tract inside the body cavity. (a) sub-terminal mouth, (b) gill arches, (c) pharyngeal teeth, (d) esophagus, (e) swim bladder, (f) stomach, (g) pyloric ceca, (h) intestine, and (i) anus.

Ecology

Little is known regarding the specifics of preferred spawning locations or spawning behavior; however, the presence of juvenile pompano throughout the spring, summer, and fall indicates a prolonged spawning season (Finucane 1969). Like many marine fishes, pompano eggs are numerous (420,000-630,000 per female) and small (0.7-0.9 mm diameter) (Moe et al. 1968, Finucane 1969). The larval period lasts about one month after hatching (Fields 1962, Finucane 1969, Gilbert 1986), during which cohorts of juveniles (10 mm TL) arrive in the surf zone of low-energy sandy beaches where they can easily be caught in large numbers with a seine (Tabb et al. 1969, Modde and Ross 1981). Upon arriving in the surf zone, juvenile pompano become opportunistic, diurnal grazers of diverse pelagic and benthic marine organisms (Finucane 1969, Bellinger and Avault 1971, Modde and Ross 1983). Juvenile fish leave the surf zone when they reach 60-80 mm TL in Georgia (Fields 1962), 110-120 mm TL in Louisiana (Bellinger and Avault 1970), 150 mm TL in Florida (Iverson and Berry 1969), or when the water temperature drops below 19^{o}C (Fields 1962), at which time they migrate into deeper, offshore waters. As adults they remain associated with coastal areas, feeding extensively in inlets and the surf zone, moving in and out with the tide (Jordan and Evermann 1905). Adult pompano are selective grazers of a large variety of polychaetes, shrimp, crabs, mussels, clams, and fishes (Iversen and Berry 1969, Finucane 1969). They can often be seen rooting or digging for food in the sand or mud, with their caudal fins sometimes appearing above the water (Jordan and Evermann 1905). Despite being classified as an estuarine species, mature adults are known to occur at depths up to 60 m (Gilbert 2002).

Fishery

Florida pompano are an important recreational fish, renowned for their tenacity on light tackle and acrobatic aerial displays. The Fisheries Statistics Division of the National Oceanic and Atmospheric Administration (NOAA) has compiled, and made available on its website (www.st.nmfs.noaa.gov), a large amount of recreational and commercial catch data for pompano. Since 1999, the recreational harvest of pompano has surpassed the commercial harvest in terms of total catch biomass (Figure 1.7). The commercial pompano fishery is a small yet important fishery, which supplies some of the finest restaurants in the Gulf of Mexico region with the staple ingredient of signature dishes (e.g., 'pompano et papillote'). The commercial catch is dominated by the State of Florida (Figure 1.8), which makes a regulatory distinction between the Gulf-coast and Atlantic-coast catches. Observed declines exhibited during the late 1970s were predominately from Gulf-coast catches; however, the continued downtrend in total commercial catch from both coasts began to point at an over-exploited fishery (Muller et al. 2002). As a result, Florida banned the use of entangling gear (i.e., gill nets) in state waters in 1996. There was an immediate increase in commercial catch of pompano on the Gulf coast. Unfortunately, subsequent declines in the commercial pompano fishery indicated that the initial increase in catch was probably the result of gill net fishermen moving to federal waters, and not a rebound in the pompano population (Muller et al. 2002).

The decline in the supply of commercially caught pompano has resulted in a steadily increasing price (Figure 1.9). During the last two decades the average ex-vessel price of pompano has consistently exceeded $6.00 per kg, with wholesale and retail prices much higher. Market demand has historically been, and continues to be, monetarily higher than a number of other commercially caught marine species (Figure 1.10). Thus economic incentive has warranted the investigation of Florida pompano as a potential aquaculture candidate.

Aquaculture

High market value and limited natural supply are the primary factors that motivated early work in pompano culture beginning in the 1950s (Berry and Iverson 1967, Bardach et al. 1972, Watanabe 1995). Florida pompano possess a number of traits desirable to aquaculture producers: good market price, adaptation to confinement and handling, fast growth, non-cannibalistic behavior, and tolerance of a wide range of environmental conditions including salinity (0-40 ppt), turbidity, water quality (pH,

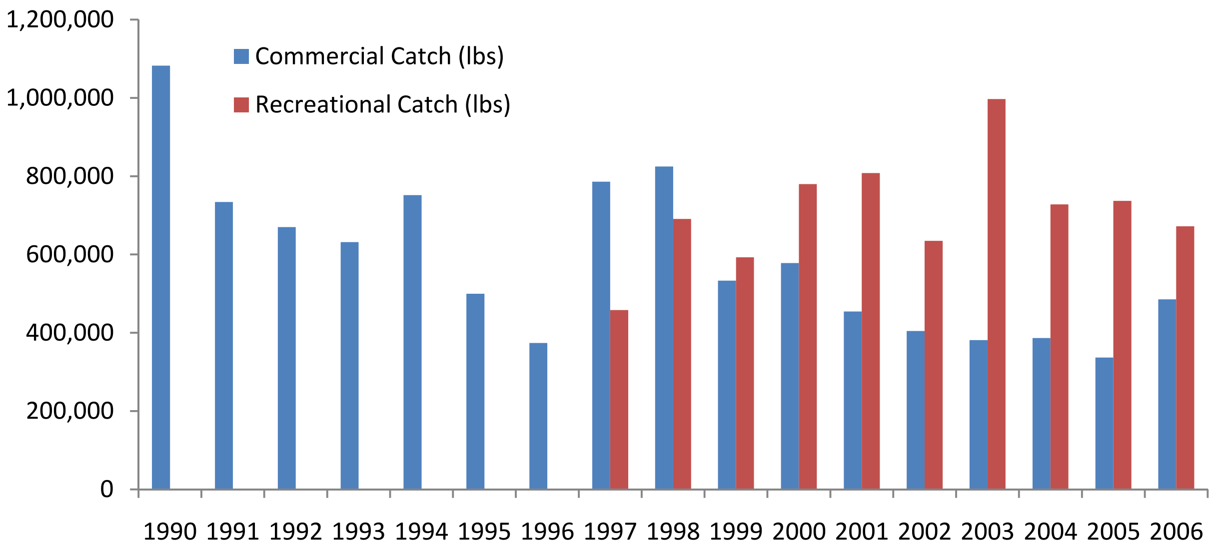

FIGURE 1.7. Annual U.S. commercial and recreational catch of pompano (Source NMFS)

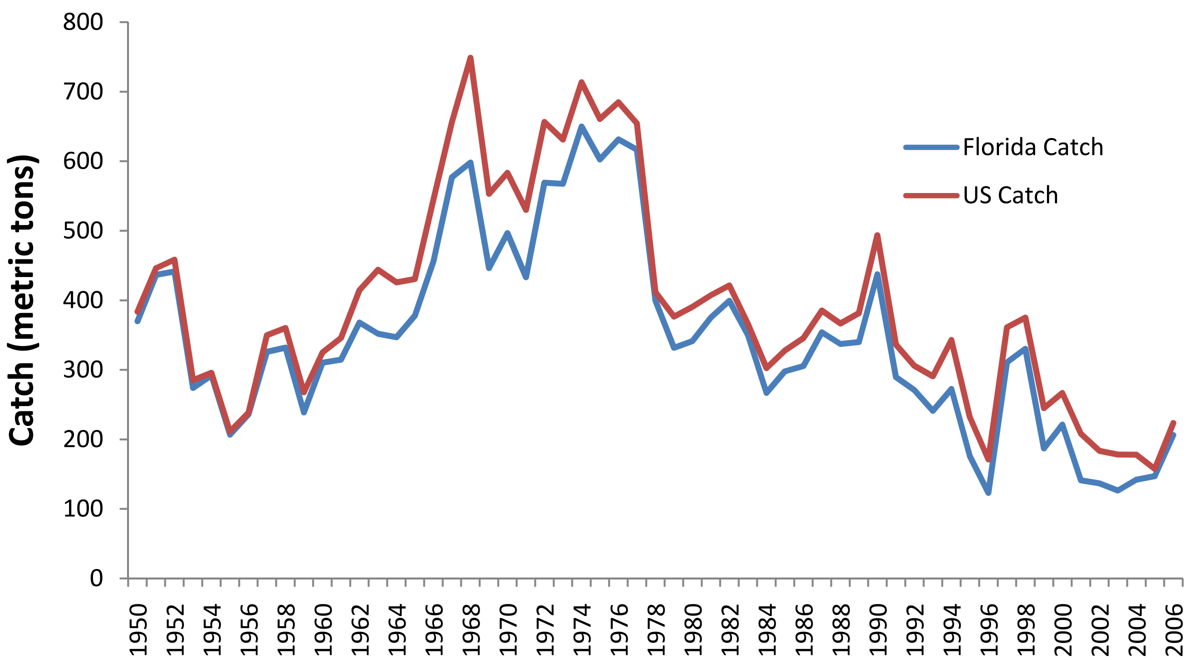

FIGURE 1.8. Annual U.S. commercial pompano catch versus annual commercial pompano catch from Florida (Source NMFS)

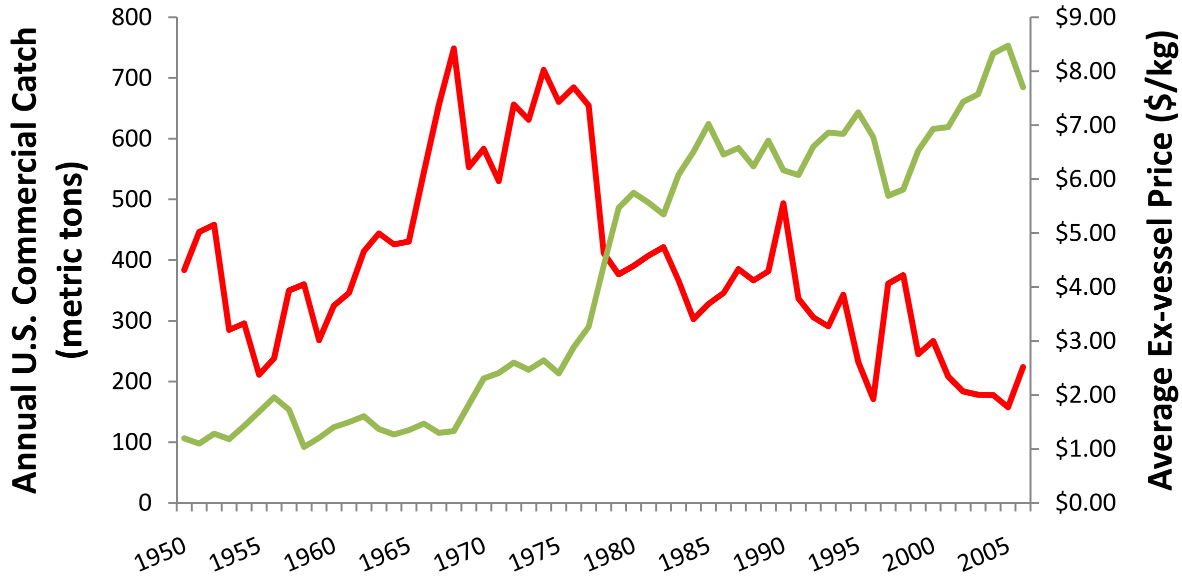

FIGURE 1.9. Annual commercial catch (red) and price (green) of Florida pompano from 1950 to 2007 (Source NMFS)

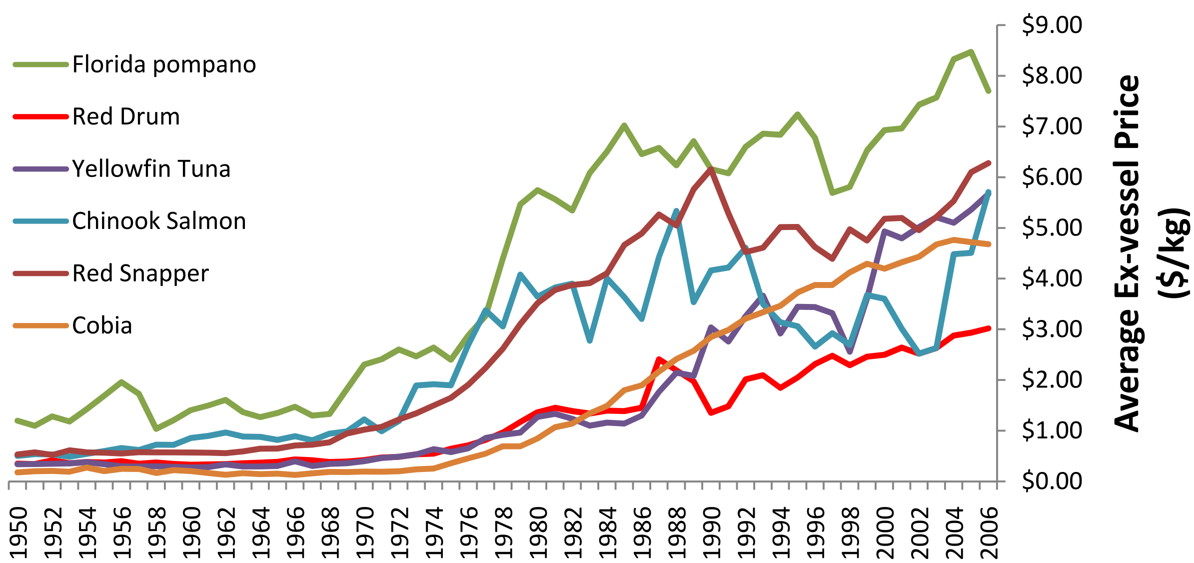

FIGURE 1.10. Annual price of pompano in relation to other commercially caught marine species (Source NMFS)

ammonia, nitrite, nitrate), and dissolved oxygen (down to 3 mg/L) (Moe et al. 1968, Finucane 1970a, Jory et al. 1985, Gilbert 1986, Tucker and Jory 1991, Pierce et al. 1993, Watanabe 1995, Stickney 1996, Weirich and Riche 2006). It was quickly identified that the major problems for pompano culture include juvenile supply, disease, water temperature, and diet (Moe et al. 1968; Finucane 1970a,b).

The majority of aquaculture research with pompano has utilized wild-caught juveniles, because of their availability and relative ease of capture. Wild fish provided an abundant, albeit seasonal, supply of pompano which have been used in a wide range of studies involving pond culture (Berry and Iverson 1967, Moe et al. 1968, Finucane 1970b, Tatum and Trimble 1978, Trimble 1980, Rossberg and Strawn 1980a,b), tank culture (Iverson and Berry 1969, Gomez and Scelzo 1982, Heilman and Spieler 1999), cage culture (Finucane 1969, Swingle 1972, Tatum 1972, Smith 1973, Holt and Strawn 1977, Jory et al. 1985), polyculture (Finucane 1970b; Rossberg and Strawn 1980a,b; Trimble 1980; Gomez and Scelzo 1982), and more recently culture in recirculation systems (Williams et al. 1985, Lazo et al. 1998, Weirich et al. 2006). A problem associated with the use of wild-caught juveniles was naturally occurring parasites (e.g., *Amlyoodinium occelatum*, *Trichodina* spp., and monogenetic trematodes) and bacterial diseases (e.g., *Mycobacterium marinum* and *Vibrio* spp.), some of which contributed to poor survival in a number of studies (Finucane 1969, Tabb et al. 1969, Gomez and Scelzo 1982, Jory et al. 1985, Watanabe 1995). Additionally, regulations restricting the capture of wild juveniles for commercial aquaculture purposes made fingerling production a limiting factor (Finucane 1970b, McMaster 1988, Avault 1996). As a result, work has been done during the last 40 years to successfully propagate pompano with a variety of techniques (Finucane 1970a,b; Hoff et al. 1972; 1978a,b; Kloth 1980; McMaster 1988; Tucker and Jory 1991; Weirich and Riley 2007).

A major problem limiting the widespread culture of pompano is their lack of cold tolerance (Berry and Iverson 1967; Finucane 1970a,b; Avault 1996). Pompano is a warmwater fish that grows best at water temperatures above 25^{o}C (Finucane 1970a,b; Watanabe 1995; Avault 1996). As temperature nears 12^{o}C pompano stop feeding and become stressed, and a water temperature of 10^{o}C is usually fatal (Moe et al. 1968; Finucane 1970a,b). Rapid temperature drops associated with cold fronts drive wild fish to deeper water, and in some cases kill those unable to find refuge (Jordan and Evermann 1905). For this reason overwintering cultured pompano in earthen ponds or cages can be accomplished only in areas without low temperature extremes. New technologies, such as indoor and greenhouse-based, closed recirculation systems, allow for much greater control of temperature and other water quality variables making year-round culture of pompano feasible in non-tropical climates.

Finucane (1970a) pointed out that a diet specifically formulated for pompano was needed; yet for years pompano nutritional research was virtually non-existent. Diets used in early feeding trials were not ideal for pompano, and results tended to be poor. Pompano were fed ground fish (Iverson and Berry 1969, Finucane 1970a, Tatum 1972, Smith 1973, Cuevas 1978), commercial by-catch (Berry and Iverson 1967, Moe et al. 1968, Jory et al. 1985), laboratory prepared diets (Gomez and Scelzo 1982), or any commercial diet available (Iverson and Berry 1969; Finucane 1970a,b; Swingle 1972; Tatum 1972; Smith 1973; Holt and Strawn 1977; Tatum and Trimble 1978; Trimble 1980). As overall knowledge of nutritional requirements for other fish species increased, better diets for marine species have been developed and these have been used in pompano growth trials with some success (Williams et al. 1985, Lazo et al. 1998, Heilman and Spieler 1999, Weirich et al. 2006). It was noted in early feeding trials that as pompano neared 200 g body weight their growth rate and feed conversion began to slow (McMaster 1988); however, more recent research has shown that higher energy diets can overcome this problem and improve growth (Weirich et al. 2006).

While knowledge of aquaculture husbandry has grown, the dearth of nutritional information on pompano continues to prevent the formulation of a nutritionally complete, cost-effective diet for this species. Research is needed to develop strategies that increase feed utilization, as well as determining minimum dietary requirements for pompano. Feeding strategies for pompano were investigated by

Heilman and Spieler (1999) who used wild-caught juveniles in culture tanks to determine circadian feeding rhythms. They found that captive wild-caught juveniles, given continuous access to food via demand bars, fed almost exclusively (99.0%) during the day, with highest feeding activity in the morning, and progressively less activity during the remainder of the day. Their finding is similar to a field study by Modde and Ross (1983) that reported the stomach volume of wild juvenile pompano increased throughout the morning and peaked in the early afternoon. Heilman and Spieler (1999) also investigated the growth effects of feeding pompano at their preferred feeding time (0600-0800 hr) versus a less preferred time (1800-2000 hr). Captive wild-caught juveniles were hand-fed trout chow at 4% of their body weight (1% per 30 min) during either the early morning period or the evening hours, for a period of 5 weeks. Fish fed in the morning had significantly lower body weight, shorter body length, and less efficient feed conversion than fish fed in the evening, indicating that feeding schedule can affect pompano growth performance. This study reports that evening-fed fish channeled more of their food intake into growth than morning-fed fish, suggesting that the preferred feeding time of pompano may not be the optimum feeding time to achieve maximum growth if pompano are only fed once per day.

Weirich et al. (2006) fed pompano a commercial diet (53% CP, 13% lipid) for 38 d to determine the effects of feeding a fixed ration (5% bw/d) in one, two, three, or six feedings per day, and found that fish fed once per day had significantly lower mean body weight. A second experiment determined that pompano fed twice daily to apparent satiation were significantly larger than fish fed a fixed ration of 5% bw/d, regardless of initial stocking density (1.3 or 2.6 kg/m^3). A third experiment determined that fish fed to apparent satiation four times daily had significantly higher mean body weight than fish fed to satiation twice daily. They reported that juvenile pompano grew from 17 g to market size (450 g) in 4-5 months and to weights in excess of 700 g in 8-9 months, with dressed carcass yields greater than 70% and fillet yields greater than 45%.

The first experiment to systematically determine pompano nutritional requirements was performed by Williams et al. (1985). They reported that the optimum level of fish oil in a 42% protein (fish meal and soybean meal) diet for juvenile pompano was 4-8% of the diet. Absence of fish oil resulted in poor growth and survival, and led to gross deformities of the gill operculum, while a 12% inclusion level resulted in lower weight gain and lower protein gain. Additionally, the researchers used 150-450 g fish to determine the digestibility of crude protein, carbohydrate, fat, and gross energy in four feedstuffs (defatted fish meal, defatted soybean meal, fish oil, and corn meal). Fecal collection was performed by inserting a catheter 10 cm from the posterior end of the digestive tract, which by their estimate was one-third of the distance from the rectum to the stomach. Chromic oxide was used in the diet as the indigestible indicator for measuring digestibility of nutrients by the *indirect method* (Cho et al. 1982). The apparent digestibility coefficient for crude protein (ACPD) in defatted fish meal and soybean meal was 86.1% and 79%, respectively. The ADC for available carbohydrate (ACD) was 53% for soybean meal and 50% for corn meal. The ADC for crude fat (ACFD) in fish oil was 68%. ADCs were used to calculate digestible energy values, which were determined to be 3.0 kcal/g for defatted fish meal, 2.7 kcal/g for defatted soybean meal, 6.1 kcal/g for fish oil, and 2.1 kcal/g for corn meal. Additionally, the researchers applied these values to their growth data (which examined the value of menhaden oil in diets for pompano) and estimated an optimum digestible energy level of 2.5-2.7 kcal/g diet in a 42% CP diet. This amount of DE implies an optimum dietary DE/DP ratio for juvenile pompano of 7.4-8.1 kcal DE/g DP.

The only other experiment to examine the nutritional requirements of pompano was reported by Lazo et al. (1998), which sought to determine the effects of dietary protein level on growth, feed efficiency, and survival of juvenile pompano. They fed four isoenergetic diets containing graded levels of protein (30%, 35%, 40%, and 45% CP) during a seven-week period. Although total feed intake was not significantly different among treatments, fish fed the highest protein diet had significantly lower daily feed consumption per unit of body weight than fish fed the lowest protein diet (30% CP = 13.2% of body weight, 45% CP = 9.1% of body weight). Growth and feed efficiency ratios increased with dietary protein level, and were highest among fish fed the 45% CP diet (weight gain = 26.2 g, FCE = 51.4). There was no dietary effect on survival (100% in all treatments). They concluded that juvenile pompano require a minimum dietary protein level of at least 45%. However, an absolute minimum dietary protein level could not be determined because a growth plateau did not occur (i.e. 'broken line' technique; Zeitoun et al. 1973, Robbins et al. 2006) at the highest dietary protein level.

CHAPTER 2

DIGESTIBILITY TRIALS TO DETERMINE NUTRIENT AVAILABILITY IN COMMON FEEDSTUFFS FOR FLORIDA POMPANO

Introduction and Objective

Florida pompano have a short gut retention time, so diets formulated for pompano should have readily available nutrients to maximize feed efficiency. The first step to properly formulate nutrient-complete, cost-effective diets for pompano is to identify the available nutrients in various feedstuffs. The easiest way to determine nutrient availability is with the *indirect method*, which uses an indigestible marker in the feed (Cho et al. 1982). Fish are fed a diet of known composition, after which a fecal material sample is collected to determine the amount of nutrients remaining. The apparent digestibility coefficient (ADC) of the diet or ingredient tested can be determined from the difference between the feed and fecal concentration of the nutrient of interest.

There are four basic methods of collecting fecal material for determining apparent digestibility (Hardy and Barrows 2002), each with drawbacks. The most precise method is to dissect each fish to remove fecal material in the posterior section of the intestine; however, this is the least desirable method because each fish can be used only once. A common collection method is aspiration, or suction, from the rectum, which can be combined with the use of a catheter. The risk associated with this technique is the collection of undigested material leading to an underestimation of digestibility. Another common method of fecal collection is tank collection, where feces are collected after elimination into the culture system, which can be performed with a siphon tube or by use of a settling column. This method is not stressful to the fish, but contact with the water allows nutrients to leach from the feces, potentially causing overestimation of digestibility. The fourth fecal collection method is manual stripping, typically by abdominal compression. The abdominal compression method involves application of gentle pressure to the lower abdominal cavity near the anus. While the manual stripping method allows repeated collections with the same fish, and avoidance of nutrient leaching, collection of undigested materials can lead to an underestimation of digestibility.

Care must be taken to ensure that the chosen technique is appropriate for the species in question. Initial observations of juvenile pompano in the laboratory indicated that fecal material began appearing 3 hr post-feeding and continued until 6 hr post-feeding. The fecal material of these pompano was in a semi-solid state. A study by Smith et al. (1980) indicated that a significant amount of the fecal nitrogen from rainbow trout was in liquid form and could leach out of the feces into the water prior to collection. For this reason we decided to collect pompano fecal material via abdominal compression during the time period 3-6 hr post-feeding.

Prior to this study, Williams et al. (1985) was the only published experiment that examined the digestibility of feedstuffs for Florida pompano (see page 16). The objective of this experiment was to build upon previous research by determining the apparent digestibility of protein, energy, and amino acids in a range of common feedstuffs of diverse chemical composition (dehulled soybean meal, menhaden fish meal, meat and bone meal, and corn grain) in order to formulate least-costs diets for Florida pompano.

Materials and Methods

Wild, juvenile pompano were collected from the surf zone of Grand Isle, Louisiana, and quarantined for at least two months at Louisiana Universities Marine Consortium (LUMCON, Cocodrie, Louisiana) (Appendix B). After the quarantine period, fish were transported to the LSU Aquaculture Research Station (LSU ARS) in Baton Rouge, Louisiana, and raised to a size suitable for digestibility trials (about 50 g, based on mouth size and fecal output) with a commercial marine starter diet (Aquaxcel, Burris Mill and Feed, Franklinton, Louisiana). Once at the LSU ARS, fish were routinely checked for residual parasites. If parasites were detected the system was treated with 0.25 mg/L chelated copper (Cutrine-Plus, Applied Biochemists, Germantown, Wisconsin), and fish were subjected to formalin baths (1 hr at 150 ppm). Prior to each digestibility trial, pompano were randomly distributed among four 600-L tanks in a recirculation system consisting of a bead filter, UV sterilizer, pump, and regenerative blower for supplemental aeration.

During the course of the digestibility trials, water quality (ammonia, nitrite, pH, alkalinity, DO, temperature, and salinity) was measured, and the systems were maintained within ranges for warm-water, mesotrophic, fingerling growout (Malone and Pfeiffer 2006). Ammonia (0-0.3 mg/L) and nitrite (0-0.5 mg/L) were below the lethal concentrations reported by Weirich and Riche (2006). The pH of the systems was 7.5-8.5; alkalinity stayed above 100 mg/L; and DO was above 5 mg/L. Water temperature ranged from 27-34°C depending on the time of year. Salinity was maintained at 10-15 ppt with synthetic sea salt (Crystal Sea, Marine Enterprises International, Baltimore, Maryland). Lighting was maintained at a 12 hr photoperiod with fluorescent lights on timers.

After the fish were distributed, tanks were randomly assigned either the reference diet (two tanks) or a test diet (two tanks). Fish were acclimated to the diets for two weeks prior to the start of each digestibility trial. The experimental diets were computer-formulated with Mixit-Win (Agricultural Software Consultants, San Diego, California) so that the test diet contained 70% of the reference diet + 30% of the test ingredient (Table 2.1). All diets contained an indigestible indicator (chromic oxide) to facilitate digestibility calculations (Cho et al. 1982). Briefly, the diets were prepared as follows. Ingredients were ground to ≤ 0.6 mm particle size with a Thomas-Wiley Laboratory Mill (Thomas Scientific, Swedesboro, New Jersey). Dry ingredients were mixed for 30 min in a twin-shell dry blender (Patterson-Kelley Co., East Stroudsburg, Pennsylvania), and transferred to a commercial food mixer (Hobart, Rapids Machinery Co., Troy, Ohio) where water, oil, and fat-soluble vitamins were added. After mixing for an additional 30 min, the wet mash was pelleted through a 1/2 HP commercial meat grinder (General Slicing, Murfreesboro, Tennessee) fitted with a 0.32-cm die. The finished diets were air-dried with electric fans for 24 hr, broken into appropriate length particles, and stored in air-tight plastic bags at -20°C.

Table 2.1. Composition of the experimental diets used for the Florida pompano digestibility trials. Test diets were comprised of 70% of the reference diet plus 30% of the test ingredient (SM = soybean meal, MB = meat and bone meal, FM = fish meal, CG = corn grain).

	Diets				
Ingredient (%, as fed)	Reference	SM-Test	MB-Test	FM-Test	CG-Test
Corn grain	20.00	14.00	14.00	14.00	*44.00*
Cottonseed meal	10.00	7.00	7.00	7.00	7.00
Fish meal, menhaden	20.00	14.00	14.00	*44.00*	14.00
Meat & bone meal, porcine	10.00	7.00	*37.00*	7.00	7.00
Soybean meal, dehulled	20.00	*44.00*	14.00	14.00	14.00
Wheat middlings	11.50	8.05	8.05	8.05	8.05
Carboxymethylcellulose	1.00	0.70	0.70	0.70	0.70
Mineral mix	1.00	0.70	0.70	0.70	0.70
Water-soluble vitamin mix	0.99	0.69	0.69	0.69	0.69
Chromic oxide	0.50	0.35	0.35	0.35	0.35
Vegetable oil	4.91	3.44	3.44	3.44	3.44
Lipid-soluble vitamin mix	0.10	0.07	0.07	0.07	0.07
Total	*100.00*	*100.00*	*100.00*	*100.00*	*100.00*
Nutrient analyzed (%, dry weight)					
Gross Energy (kcal/g diet)	4.61	4.57	4.61	4.64	4.35
Crude Protein (%)	38.28	42.35	46.01	47.91	29.68
Chromic oxide (%)	0.51	0.41	0.36	0.32	0.31

Digestibility trials were run sequentially, so the size and number of fish varied (Table 2.2). Diets were fed to apparent satiation to duplicate groups of pompano twice daily, and fecal samples were collected weekly by abdominal compression. On the fecal collection day, fish in all four tanks were fed initially. Beginning 30 min after the initial feeding, and according to a randomly assigned sampling order, fish in each tank were fed a second time during staggered 30 min intervals. This ensured that all fish were being sampled 3 hr after the second feeding and between 3.5 and 5 hr after the first feeding. This feeding/sampling strategy took advantage of the 3-6 hr fecal evacuation period observed in the laboratory, and ensured that some fecal material remained in the intestine when it came time to strip individual fish (in some trials over 100 fish were sampled per tank). Fish were quickly removed from the culture tank, placed in a well-aerated plastic tub (20 L), and anesthetized in a solution of clove oil (200 ppm). Fish were blotted dry with paper towels, and gentle thumb pressure was applied to the lower third of the abdomen to obtain fecal samples, which was collected in aluminum drying pans. Fish were revived in a recovery tank, then transferred back to their original culture tank.

After collection, the fecal material was dried overnight at 100^{o}C , pooled by tank, and stored in air-tight plastic bags at -20^{o}C until analyzed. When a total of approximately 5 g of dried feces per tank had been collected, the fecal material and feed samples were ground with a mortar and pestle and analyzed in triplicate. Samples were submitted to the LSU Agricultural Chemistry Department to determine the chromium content with inductively coupled plasma spectrometry (ICP). The remaining analyses were performed in the LSU ARS Nutrition Laboratory. Gross energy content was determined with adiabatic bomb calorimetry (Model 1241 adiabatic bomb calorimeter, Parr Instrument, Moline, Illinois), crude protein with the Kjeldahl method (Kjeltec Auto 1030 Analyzer with Digestion System 20; Tecator AB, Hoganas, Sweden), and amino acids with high pressure liquid chromatography (HPLC; Agilent 1100 Series, Agilent Technologies, Wilmington, Delaware).

The nutrient content of the reference diet, test diets, and fecal samples was used to calculate

TABLE 2.2. Information on juvenile pompano involved in the digestibility trials. The first column is the date that the juveniles were collected from Grand Isle, Louisiana. The second column identifies the digestibility trial in which the fish were used (soybean meal [SM], fish meal [FM], meat and bone meal [MBM], and corn grain [CG]), with the number in parenthesis being the attempt at each trial. Column three is the start date of the trial; column seven is the finish date. Those trials without a finish date are the ones that terminated prematurely (reason listed). Columns 4-6 are the mean number of fish per tank at the start of each growth trial, the mean weight (g) and mean length (cm), respectively. The final column indicates the number of fecal collections needed to obtain the desired 5 g of dried fecal material.

Date Collected	Test Ingredient	Start Date	Mean Fish/tank	Mean Wt.	Mean Lg.	Finish Date	Fecal Collections
7/15/2004	SM	5/30/2005	136.5	44.59	15.45	7/22/2005	7
7/15/2004	FM (1)	8/2/2005	44	74.86	18.33	*a*	
7/15/2004	MBM (1)	8/2/2005	61	72.74	17.04	*b*	
7/15/2004	MBM (2)	10/6/2005	20	72.74	17.04	12/30/2005	9
9/20/2005	FM (2)	3/31/2006	20.5	110.65	19.09	6/28/2006	8
9/20/2005	CG (1)	8/15/2006	13	225.69	24.23	*c*	
8/3/2006	CG (2)	1/6/2007	42	75.68	18.03	3/10/2007	7

a – During the diet acclimation period a piece of silicon lodged in the pump impeller, reducing the water flow to each tank, which caused a complete fish kill.

b – One month after starting the digestibility trial, a pipe came loose from the filter causing the majority of the water volume to be pumped out of the system. The majority of the fish were killed; however, two of the four tanks had survivors. The fish that lived were redistributed for MBM (2) once it was determined that no more fish collections from the coast could be made during late 2005 (Hurricanes Katrina and Rita).

c – We attempted to use the few fish collected (577 fish) during the 2005 sampling season for as much research as possible, which included FM (2) and CG (1). By the start of CG (1) very few fish were left, and when a few of these died there were not enough remaining to continue.

apparent digestibility coefficients (ADC) for the nutrients of interest (energy, protein, and amino acids). This calculation is based on differences between the nutrient composition of the diet and the feces, in relation to the marker, and was calculated for each experimental tank:

$$ADC_{tank} = 100 – [(indicator\ in\ feed/indicator\ in\ feces) \times (nutrient\ in\ feces/nutrient\ in\ feed) \times 100]$$

where indicator is chromic oxide and nutrient is energy, protein, or amino acids. ADC for energy, protein and amino acids in the test ingredient was calculated for each ingredient using the following formula, which is based on the 70:30 ratio of reference diet and test ingredient (Cho et al. 1982):

$$ADC_{nutrient} = (100/30) \times [ADC_{test} – (70/100) \times (ADC_{reference})]$$

Data were subjected to analysis of variance (ANOVA; SAS Institute 9.1, Cary, North Carolina) to determine the difference in mean digestibility values for the nutrients of interest in the ingredients tested (differences were considered statistically significant at $P \leq 0.05$). When differences were indicated, Tukey's Studentized range test was used to separate significantly different means.

Results and Discussion

Energy

Apparent energy digestibility (AED) coefficients ranged from 41% to 105% (Table 2.3). Energy digestibility of fish meal (FM) was higher than energy digestibility of the other ingredients tested. AED of soybean mean (SM) and meat and bone meal (MB) did not differ significantly. AED of corn grain (CG) was significantly lower than the other ingredients tested.

Carnivorous fishes tend to utilize dry matter and energy in feedstuffs of animal origin more efficiently than those of plant origin (Cho et al. 1982, Bergot and Breque 1983, Ellis and Reigh 1991, Reigh and Ellis 1992, McGoogan and Reigh 1994, Sullivan and Reigh 1995, Gaylord and Gatlin 1996, Lee 2002). While FM digestibility reflected this trend, the AED determined for MB is on the low end of values reported for other species (Table 2.4). The low AED value for MB may be due to the high ash content (37%); however, the menhaden FM used had an ash content of 20%. Processing and other factors may

Table 2.3. Apparent energy digestibility (AED), apparent crude protein digestibility (ACPD), and the range for apparent amino acid availability (AAAA) contained in various feedstuffs for Florida pompano *Trachinotus carolinus*. Values (mean ± SD) in each column that share the same letter are not significantly different ($P > 0.05$).

Ingredient	AED (%)	ACPD (%)	AAAA Range (%)
Soybean Meal	67.4 ± 0.8 b	84.3 ± 0.5 ab	78.4 ± 2.2 – 96.5 ± 1.0
Fish Meal	105.1 ± 5.4 a	95.3 ± 1.9 a	89.0 ± 7.9 – 109.1 ± 14.6
Meat and Bone Meal	65.7 ± 8.2 b	62.4 ± 6.1 c	48.1 ± 9.5 – 84.7 ± 3.8
Corn Grain	41.4 ± 0.5 c	71.4 ± 1.0 bc	40.0 ± 3.9 – 85.0 ± 0.4

TABLE 2.4. Apparent digestibility coefficients for gross energy (AED) in soybean meal (SM), fish meal (FM), meat and bone meal (MB), and corn grain (CG) for Florida pompano and other species

	AED (%)				
Species	**SM**	**FM**	**MB**	**CG**	***Citation***
Pompano	*67*	*100*	*65*	*41*	*Current study*
Pompano	60	70		49	Williams et al. (1985)[1]
Asian sea bass	69	69-83			Boonyaratpalin and Williams (2002)
Atlantic cod	88	92			Tibbetts et al. (2006)
Channel catfish	70	92	75		NRC (1993)
Channel catfish	72	92	76	57	Wilson (1991)
Channel catfish	73	95	70	79	Brown et al. (1985)
Chinook salmon	78	92			NRC (1993)
Cobia	87	95	90		Zhou et al. (2004)
European sea bass	69-70	86-97			Gomes da Silva and Oliva-Teles (1998)
European sea bass	69	86-95			Kaushik (2002)
Hybrid striped bass	55	96	80	41	Sullivan and Reigh (1995)
Rainbow trout	75	91	80	39	Bureau et al. (2002)
Rainbow trout	70		82		NRC (1993)
Rainbow trout	75	91	85	39	Cho and Cowey (1991)
Red drum	63	92	86		Gaylord and Gatlin (1996)
Red drum	37	60	54	56	McGoogan and Reigh (1996)
Yellow croaker		83	70		Ai et al. 2006
Yellowtail	62	83	82		Masumoto (2002)

[1] Digestible energy values obtained in this study were calculations for defatted fish meal, defatted soybean meal, and corn grain determined by multiplying the percentages of digestible protein, carbohydrate, and fat by 5.65, 4, and 9 kcal/g, respectively.

also have affected the ADC value for MB because the quality of animal by-product meals is known to vary with the chemical composition of the available raw materials (Hertrampf and Piedad-Pascual 2000). The AED coefficient for SM is similar to the value reported for pompano by Williams et al. (1985), and is consistent with results of previous studies on other species (Table 2.4). Of the ingredients tested, CG had the lowest AED value, which is comparable to results from other studies (Table 2.4) and is presumably caused by the high starch content (76%) of corn. Utilization of plant starch as an energy source differs among species and appears to be limited among carnivores (Cowey and Walton 1989, NRC 1993). However, partial hydrolysis of starch through thermal or hydrothermal means (e.g., cooking, gelatinization, extrusion) can enhance digestibility values, which has been shown in numerous studies (Hilton et al. 1981, Bergot and Breque 1983, Boccignone et al. 1989, Pfeffer et al. 1991, Venou et al. 2003). More research is needed to determine if hydrolyzation of high starch ingredients, like corn grain, can increase the AED, thus providing inexpensive sources of energy in diets for Florida pompano.

Protein

Apparent crude protein digestibility (ACPD) coefficients ranged from 62% to 95% (Table 2.3). Significant differences in ACPD did exist among feedstuffs, but no trend among products of animal or plant origin was apparent. FM had the highest protein digestibility, but was not significantly different than SM. MB had significantly lower protein digestibility than the other ingredients tested.

Proteins in most feedstuffs that have been properly processed are highly digestible by fishes (NRC 1993). Protein digestibility of all ingredients tested, except meat and bone meal, was high (> 70%) and compared favorably with previously reported data on pompano and other species (Table 2.5). The ACPD values obtained for FM and SM were 9% higher than the values reported in the Williams et al. (1985) pompano study, which may be due to differences in fecal collection techniques. Protein from FM is well digested by most carnivorous fishes (Table 2.5), which is consistent with results of this study. The ACPD value obtained for MB is lower than other reported values (Table 2.5), which was unexpected

TABLE 2.5. Apparent digestibility coefficients for crude protein (ACPD) in soybean meal (SM), fish meal (FM), meat and bone meal (MB), and corn grain (CG) for Florida pompano and other species

	ACPD (%)				
Species	**SM**	**FM**	**MB**	**CG**	***Citation***
Pompano	*84*	*95*	*62*	*71*	*Current study*
Pompano	79	86			Williams et al. (1985)
Asian sea bass	86	88-92			Boonyaratpalin and Williams (2002)
Atlantic cod	92	93			Tibbetts et al. (2006)
Atlantic salmon	71	78			Storebakken (2002)
Atlantic salmon	88	89			NRC (1993)
Blue tilapia		85			NRC (1993)
Channel catfish	93	88	78		NRC (1993)
Channel catfish	97	85	61	97	Wilson (1991)
Channel catfish	85	86	82	92	Brown et al. (1985)
Chinook salmon	77	83	85		NRC (1993)
Cobia	91	96	87		Zhou et al. (2004)
European sea bass	89-90	90-96			Gomes da Silva and Oliva-Teles (1998)
European sea bass	90	90-96			Kaushik (2002)
Hybrid striped bass	80	88	73	87	Sullivan and Reigh (1995)
Rainbow trout	96	92	85	95	Bureau et al. (2002)
Rainbow trout	83		82		NRC (1993)
Rainbow trout	96	92	85	95	Cho and Cowey (1991)
Red drum	86	77	79		Gaylord and Gatlin (1996)
Red drum	80	96	74	82	McGoogan and Reigh (1996)
Yellow croaker		92	82		Ai et al. 2006
Yellowtail	93	84-89	80		Masumoto (2002)

considering the high level of protein contained in MB.

Feed ingredients of plant origin often do not contain levels of protein as high as ingredients of animal origin, but protein in many plant products appears to be digested as efficiently by carnivorous and omnivorous fishes as that in animal products (Cho and Cowey 1991, Wilson 1991, Sullivan and Reigh 1995, McGoogan and Reigh 1996). The digestibility of protein in SM for pompano was similar to values reported for many other species (Table 2.5). This suggests plant protein products, like SM, might be used effectively in diets formulated for pompano. The ACPD of CG was slightly lower than values reported for rainbow trout, channel catfish, and red drum (Table 2.5) yet was still relatively high considering the high level of indigestible starch contained in CG.

Amino Acids

Apparent amino acid availability (AAAA) coefficients ranged from 40% to 109% (Table 2.3, 2.6). The AAAA values for the ingredients tested suggest a reasonable agreement between protein and amino acid digestibility; however, the availability of individual amino acids within a feed ingredient varies. Most AAAA values for FM and SM were significantly higher than the other ingredients tested (Table 2.6), but were not significantly different from each other. AAAA values of MB were the lowest among ingredients tested, except for lysine which was significantly lower for CG (Table 2.6). Determination of the availability of individual amino acids provides more information about nutrient utilization than crude protein digestibility alone.

Protein quality of feedstuffs depends not only on amino acid composition but also the availability of amino acids. Fish obtain amino acids by consuming protein; however, the amino acid content of proteins, particularly feed proteins, may differ markedly. An absolute requirement for ten amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) has been demonstrated in all fish species examined so far (NRC 1993). These essential amino acids (EAA) cannot be synthesized by fish, and must be supplied in the diet.

TABLE 2.6. Apparent amino acid availability (AAAA) of soybean meal (SM), fish meal (FM), meat and bone meal (MB), and corn grain (CG) for Florida pompano. Within rows, values (mean ± SD) with the same letter are not significantly different ($P > 0.05$).

Amino Acid	SM	FM	MB	CG
Alanine (Ala)	93.7 ± 1.6 a	102.2 ± 2.1 a	69.9 ± 2.0 b	75.0 ± 3.3 b
Arginine (Arg)	96.5 ± 1.0 a	105.0 ± 3.6 a	69.0 ± 4.1 c	81.9 ± 1.2 b
Aspartic acid (Asp)	93.6 ± 1.6 a	97.8 ± 0.1 a	48.1 ± 9.5 b	77.6 ± 3.1 a
Cystine (Cys)	84.8 ± 3.2 a	91.8 ± 10.6 a	54.1 ± 6.1 b	81.2 ± 2.6 a
Glutamic Acid (Glu)	94.3 ± 1.9 ab	103.7 ± 3.1 a	65.5 ± 7.0 c	85.1 ± 0.4 b
Glycine (Gly)	91.4 ± 1.8 a	89.0 ± 7.9 a	64.1 ± 1.0 b	71.7 ± 1.8 b
Histidine (His)	88.8 ± 6.1 ab	94.7 ± 7.4 a	73.1 ± 3.3 b	79.3 ± 2.4 ab
Hydroxyproline (Hyp)	78.4 ± 2.2 ab	109.1 ± 14.6 a	79.7 ± 19.8 ab	40.0 ± 3.9 b
Isoleucine (Ile)	94.7 ± 1.4 a	100.7 ± 1.1 a	70.8 ± 6.8 b	75.0 ± 5.5 b
Leucine (Leu)	94.2 ± 1.6 ab	101.7 ± 0.9 a	70.3 ± 6.3 c	80.6 ± 1.9 bc
Lysine (Lys)	93.4 ± 0.4 ab	106.3 ± 1.6 a	84.7 ± 3.8 b	66.6 ± 5.1 c
Methionine (Met)	94.3 ± 1.3 ab	101.9 ± 1.8 a	63.2 ± 7.1 c	70.4 ± 9.8 bc
Phenylalanine (Phe)	94.9 ± 1.5 a	98.1 ± 0.9 a	73.6 ± 7.1 b	73.2 ± 5.4 b
Proline (Pro)	91.1 ± 2.3 ab	100.1 ± 2.4 a	68.7 ± 1.1 c	80.7 ± 0.8 b
Serine (Ser)	91.1 ± 3.9 ab	102.5 ± 9.9 a	60.8 ± 7.8 c	72.0 ± 3.0 bc
Threonine (Thr)	91.1 ± 2.4 ab	103.2 ± 2.0 a	63.0 ± 10.9 c	68.5 ± 1.6 bc
Tyrosine (Tyr)	92.1 ± 3.6 a	97.1 ± 16.7 a	68.4 ± 11.3 a	69.3 ± 7.3 a
Valine (Val)	94.8 ± 1.7 a	100.5 ± 0.8 a	67.5 ± 7.4 b	72.1 ± 2.4 b

Furthermore, other amino acids can have a sparing effect on the EAA, specifically cystine for methionine and tyrosine for phenylalanine. In order to assess the quality of the protein source, the apparent essential amino acid availability (AEAAA) needs to be determined. The protein hydrolysis procedure used in our analysis destroyed tryptophan in the samples, so this amino acid is not included in the results. Additionally, the amino acids cystine and tyrosine are included because of the complementary role in meeting dietary requirements for methionine and phenylalanine, respectively.

The AEAAA values of SM and FM for pompano are generally higher than they are for both Atlantic salmon and channel catfish, and are very similar to values reported for cobia (Table 2.7). Worldwide availability, relatively low cost, and high protein content has made SM the most important protein source in feeds for farm animals and also as a partial replacement for FM in aquaculture diets (Hertrampf and Piedad-Pascual 2000). SM has one of the best amino acid profiles of all oilseed meals; however, limiting amino acids are methionine and cystine. For pompano the availability of methionine in SM was quite high, yet arginine was the highest determined value. The availability of EAA in FM was exceptionally high for pompano, with many AA being essentially 100% available. The AEAAA values of MB, however, were very low for pompano, much lower than has been reported for cobia and catfish, reinforcing the possibility that processing or other ingredient-related factors affected this outcome. Although not a major protein source, the AEAAA values of CG were numerically lower for pompano than for catfish.

A comparison of the proteinaceous ingredients investigated in this digestibility trial (SM, FM, MB) elucidates the importance of selecting feedstuffs for nutrient availability, not just for chemical composition. The CP level (as analyzed on an as-fed basis) of SM was 49%, compared with 62% CP for FM, and 58% CP for MB. Comparisons of these ingredients based on CP levels would be crude at best. For the purpose of this discussion, ingredients will be compared by the sum (%) of the EAA (plus cystine and tyrosine; without tryptophan, which could not be detected in the samples analyzed in the

TABLE 2.7. Apparent essential amino acid availability (AEAAA), of soybean meal (SM), fish meal (FM), meat and bone meal (MB), and corn grain (CG) for Florida pompano (this study), Atlantic salmon ([1]NRC 1993, [2]Storebakken 2002), channel catfish (NRC 1993), and cobia (Zhou et al. 2004).

	AEAAA (%)										
SM	ARG	CYS	HIS	ILE	LEU	LYS	MET	PHE	THR	TYR	VAL
Pompano	*96.5*	*84.8*	*88.8*	*94.7*	*94.2*	*93.4*	*94.3*	*94.9*	*91.1*	*92.1*	*94.8*
A. salmon[1]	86.7		86.4	79.2	75.9	83.6	94.0	78.7	84.7	83.0	77.3
A. salmon[2]	77		77	67	70	67	71	70	62	68	67
C. catfish	96.8		87.9	79.7	83.5	94.1	84.6	84.2	82.2	83.3	78.5
Cobia	92.8		89.2	90.6	91.2	93.4	92.1	90.2	90.3	90.3	89.4
FM											
Pompano	*100*	*91.8*	*94.7*	*100*	*100*	*100*	*100*	*98.1*	*100*	*97.1*	*100*
A. salmon[1]	86.8	92.0	91.1	88.5	90.1	87.6	83.6	87.4	88.4	92.1	86.3
A. salmon[2]	91		85	91	89	84		89	85	86	90
C. catfish	91.0		84.5	87.1	89.0	86.4	83.1	87.3	87.4	88.8	87.1
Cobia	97.6		97.4	96.8	96.7	97.5	95.9	95.2	96.6	96.6	95.2
MB											
Pompano	*69.0*	*54.1*	*73.1*	*70.8*	*70.2*	*84.7*	*63.2*	*73.6*	*63.0*	*68.4*	*67.5*
C. catfish	87.9		82.2	80.8	82.4	86.7	80.4	85.4	76.3	83.1	80.8
Cobia	93.1		88.2	91.1	92.3	84.5	92.6	91.5	91.6	91.6	91.4
CG											
Pompano	*81.9*	*81.2*	*79.3*	*75.0*	*80.6*	*66.6*	*70.4*	*73.2*	*68.5*	*69.3*	*72.1*
C. catfish		82.0	90.3	67.9	87.5	96.5	70.5	81.8	69.8	77.5	74.4

laboratory) contained in each ingredient (ΣEAA). Comparing the EAA content of the ingredients provides more information about the utility of these feedstuffs, especially when compared to the EAA content of whole-body pompano (WBP). In the absence of AA requirements, the AA profile of whole body tissue has been used effectively as an index until specific EAA requirements are established (Wilson and Poe 1985, Wilson 2002). Amino acid analyses and crude protein analyses were performed on multiple samples of pompano, and are presented in Table 2.8.

The absolute amino acid content and CP values for WBP of small fish (mean weight 1.7 g) were slightly higher than pompano muscle and much higher than WBP values of larger fish (mean weight 17 g and 34 g). However, when the ΣEAA values are adjusted for CP content of the samples (ΣEAA /CP), the resultant values of 0.34-0.38 are much more uniform, and indicate roughly 34-38% of CP in WBP is composed of EAA (Table 2.8). The ΣEAA for the protein sources reveals that EAA comprise roughly 40%, 53%, and 46% of CP in SM, FM, and MB, respectively (Figure 2.1). These values are slightly higher than the mean value of 36% for WBP (Figure 2.1). Adjustments for CP in each sample reveal the relative EAA content to be highest for FM and lowest for SM (Figure 2.1).

Using the apparent essential amino acid availability (AEAAA) values for the ingredients, compared to the EAA content of WBP, determinations can be made regarding the biological value of protein sources for Florida pompano (Figure 2.2). The protein score (PS *= (Σ EAA in ingredient) / (Σ EAA in WBP)*) for SM was 0.64, meaning SM contains roughly 60% of the essential amino acids (EAA) found in whole body pompano. The PS for FM was 1.07, equivalent to whole body pompano. The PS for MB was 0.87. This PS indicates that both FM and MB are likely to be better protein sources than SM; however, by correcting for AEAAA the hierarchy changes (Figure 2.2). Corrected protein score (CPS *= [Σ ((EAA x ADC) / 100)] / (Σ EAA in WBP)*) values were 0.60, 1.05, and 0.57 for SM, FM, and MB, respectively. The CPS of FM is still equivalent to WBP, yet the value for MB drops below SM. This reduction in comparative protein score for MB is an important consideration when choosing protein sources. While

TABLE 2.8. Body composition analysis of Florida pompano. The top section includes date fish were collected by beach seine in Grand Isle, Louisiana. Analyzed samples were pooled and consisted of the listed number of fish, which were various average sizes (and ages from collection). The first batch of fish was oven-dried (OD), while the others were freeze-dried (FD) for measurement of dry matter (%). Samples A - D were whole-body juvenile pompano, while E was the fillet of a large pompano. Crude protein (%) and individual amino acid content (%) are expressed on a dry weight basis. The sum of the essential amino acids (ΣEAA) is listed, excluding tryptophan (not measured), and including cystine and tyrosine.

Date collected	7/15/2004	5/13/2006	10/6/2006	4/10/2007	9/20/2005
Number of fish	roughly 20-50	34	5	3	1
Avg. size of fish	roughly 1-3 g	1.7 g	16.8 g	34.3 g	roughly 350 g
Procedure	OD	FD	FD	FD	FD
Analysis	whole-body	whole-body	whole-body	whole-body	fillet
Dry matter	unknown	20.5%	33.9%	30.0%	32.7%
Crude protein	73.2%	73.7%	47.3%	49.8%	59.8%
Amino acid	*A*	*B*	*C*	*D*	*E*
Arginine	2.58	2.70	1.81	1.77	2.12
Cystine	2.36	2.12	1.41	1.55	1.17
Histidine	0.93	1.02	0.66	0.65	0.98
Isoleucine	2.39	2.37	1.71	1.60	2.42
Leucine	3.87	4.02	2.79	2.62	3.76
Lysine	4.47	3.69	3.04	2.62	3.85
Methionine	1.48	1.45	0.91	0.87	1.27
Phenylalanine	1.74	1.72	1.18	1.10	1.44
Threonine	2.17	2.43	1.58	1.54	2.05
Tyrosine	0.89	0.96	0.45	0.45	0.74
Valine	2.87	3.10	2.25	2.11	2.99
ΣEAA	*25.73*	*25.58*	*17.80*	*16.88*	*22.78*

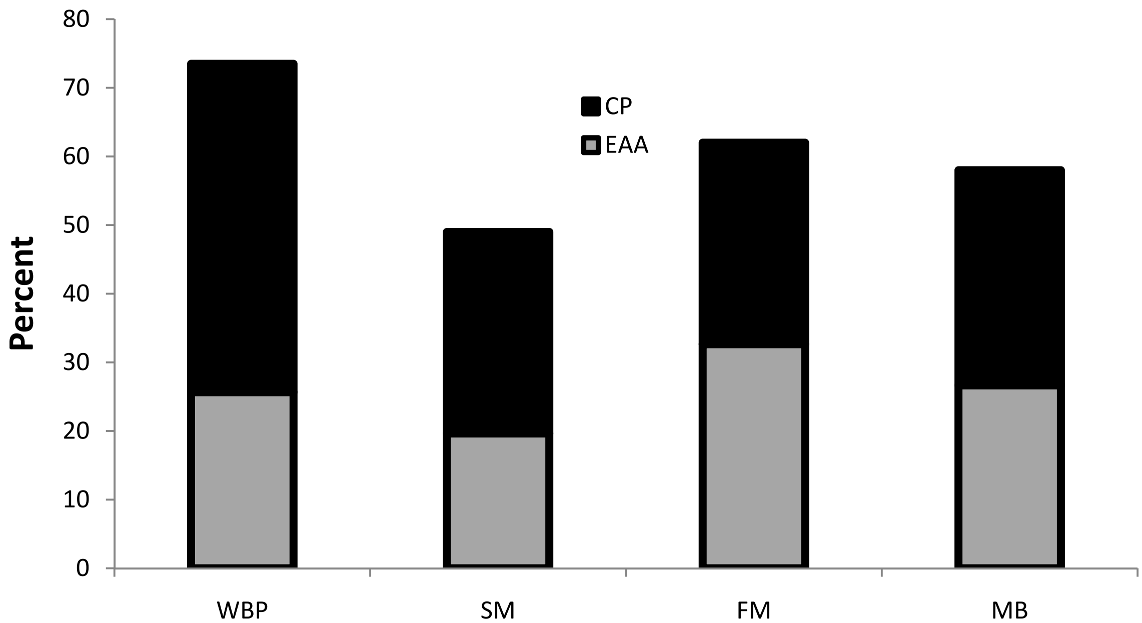

FIGURE 2.1. Comparison of the crude protein (CP) levels (%) of whole-body pompano (WBP), soybean meal (SM), fish meal (FM), and meat and bone meal (MB), and the relative content (%) of essential amino acids (EAA) contained in each. Vertical bars represent CP level and lighter areas represent the proportionate EAA content.

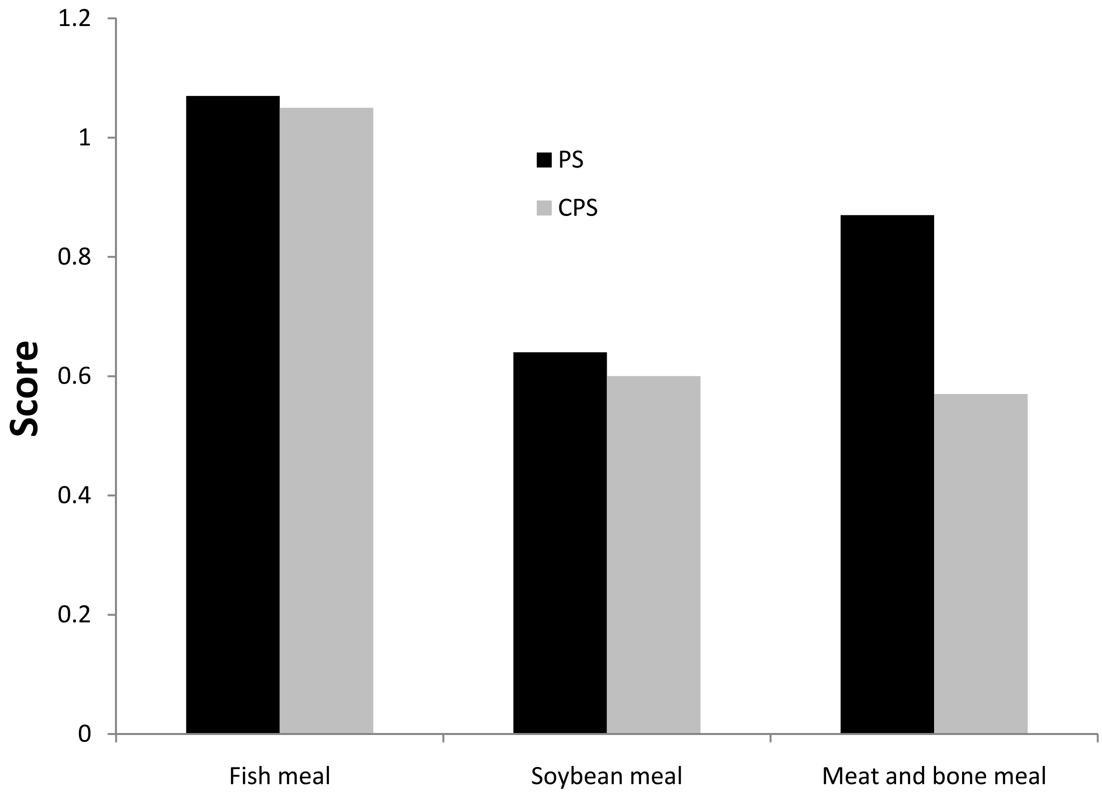

FIGURE 2.2. Protein score (PS) of various feedstuffs for Florida pompano determined by comparing the sum of the essential amino acids (EAA) in whole-body juvenile pompano (WBP) to the sum of the EAA contained in the ingredient. This value is corrected for the individual amino acid availability of the various ingredients (CPS). A value of 1.0 indicates an EAA profile similar to WBP, while values lower than 1.0 are inferior to WBP in terms of EAA profile.

the animal derived protein source would be expected to be better, the plant derived protein source actually appears to be better because of its higher amino acid availability.

The digestibility results discussed here are only the beginning of nutritional work that needs to be done with pompano. It is important to note that the ability to investigate amino acid availability, in addition to CP digestibility, provides a much more insightful evaluation of feedstuffs. This is because the value of a protein depends upon the amino acids it contains and their availability. As discussed regarding MB, the presence of a large quantity of EAA does not necessarily result in a superior protein source. These data provide preliminary background information that can be utilized to formulate nutritionally complete diets for pompano; however, in order to formulate least cost diets the digestibility coefficients must be determined on a wider range of ingredients. Despite the superior quality of fish meal as a protein source in aquaculture diets, the high cost of fish meal and questions concerning its sustainable production requires that alternative protein sources be identified and evaluated. Once this information is obtained and combined with the nutritional requirements of pompano, a diet can be formulated to meet the high protein, high energy demands of this desirable species.

Conclusions

Apparent digestibility coefficients of feed ingredients provide information concerning nutrient utilization, which can enable least-cost formulation of diets designed for Florida pompano. An advantage in using apparent digestibility values is that these values are additive, which means the nutrient value of a diet as a whole can be calculated based on the individual ingredients (Cho et al. 1982). Nutrient digestibility varies with the chemical composition of feedstuffs used and the physiology of the species. Natural diets of carnivorous fishes contain high levels of fat and protein and very little carbohydrate (Smith 1989). As a result, utilization of dietary energy by carnivorous fishes tend to be negatively related to the carbohydrate content of the diet and positively related to dietary lipid and protein content (Sullivan and Reigh 1995). The carnivorous nature of pompano is evident in the gastrointestinal tract morphology, and the pompano's ability to easily digest fish meal in this study is consistent with its wild diet of fishes, crustaceans, and mollusks.

This study has shown that Florida pompano digested more energy from high protein ingredients than from ingredients containing high levels of carbohydrate. However further investigation is warranted to determine if carbohydrate digestibility can be improved in order to increase the use of starchy ingredients in diet formulations. Protein digestibility was high in feedstuffs of plant and animal origin, as was the apparent availability of EAA. As the body of pompano nutrition knowledge grows, protein sources for pompano may be selected primarily on the basis of amino acid availability and price. Because essential amino acid availabilities have not been reported for pompano, use of amino acid data obtained from this study should allow for more accurate and economical pompano feed formulation.

CHAPTER 3
GROWTH OF FLORIDA POMPANO FED DIETS CONTAINING GRADED LEVELS OF PROTEIN AT A FIXED ENERGY-TO-PROTEIN RATIO

Introduction and Objective

Florida pompano are highly active carnivorous fish that swim constantly. These biological factors are important considerations when attempting to formulate pompano diets. The active nature of pompano indicates a need for high levels of digestible energy. The natural diet and short gut of pompano warrants formulated diets that contain high levels of easily digestible nutrients. Protein is typically the most expensive component in aquaculture diets, thus dietary protein levels directly affect production costs. Currently little is known about the optimum dietary protein level for juvenile pompano. The objective of this research was to investigate the growth effects of varying protein levels in diets for pompano that contain a fixed energy-to-protein ratio.

Animals, including fishes, do not have protein requirements but rather requirements for specific amino acids. Although dietary protein requirements have been measured and reported for a number of species, these values are estimates made from dose-response curves yielding the minimum amount of dietary protein that resulted in maximum growth (Wilson 2002). Protein requirement values obtained by this method may not be accurate because of one or more of the following dietary factors: (a) the energy concentration of the diet, (b) the amino acid composition of the dietary protein, and (c) the digestibility of the dietary protein (Wilson 2002). When undertaking protein requirement investigations, it is important to take these dietary factors into consideration.

It is possible that different protein levels may be optimum as protein sources in a diet change and the energy needs of the fish vary. The level of dietary energy in a diet can influence changes in protein utilization as well as protein sparing effects (Wilson 1989). It has also been suggested that fishes, like other animals, eat to meet their energy requirements (Smith 1989, Bureau et al. 2002), so diets with high levels of energy may result in fish halting consumption before satisfying their protein needs.

Inadequate protein in the diet results in a reduction of growth and loss of weight, while too much protein in the diet results in only part of the protein consumed being used for growth with the remainder converted to energy (Wilson 2002). These factors make quantification of dietary protein requirements problematic. By using experimental diets containing a fixed amount of energy (isoenergetic), the growth effects of varying levels of protein can be tested; however, the diets containing the highest levels of protein may provide insufficient energy to optimize protein utilization. The result would be expensive protein used as an inefficient energy source. A preferable method to test optimal dietary protein levels is to maintain a consistent energy-to-protein ratio. This means that for every gram of protein included in the diet, a corresponding amount of energy would also be provided. Diets with appropriate energy-to-protein ratios will maximize protein-sparing effects, and reduce the cost of gain by minimizing the amount of dietary protein that is used for maintenance.

To date, a single study has reported the protein requirement of Florida pompano to be at least 45% of a diet in which soybean meal and fish meal were the major protein sources (Lazo et al. 1998). This conclusion was based on maximum growth (26.2 g weight gain) and feed conversion efficiency (51.4%) over a seven-week period. However, 45% CP was the highest protein level tested. As no growth plateau was observed with regard to various protein levels, a minimum dietary protein requirement could not be determined by the 'broken-line' technique (Zeitoun et al. 1973, Robbins et al. 2006). The researchers used isoenergetic diets, and concluded that the lack of significant differences in protein conversion efficiency (17.6- 19.7%) and protein efficiency ratio (0.9-1.5) indicated energy was not limiting with respect to protein. Proximate compositions of the fish indicate the energy-to-protein ratio of the diets (8.9-13.2 kcal/g CP) was adequate. Percent daily feed consumption (PDFC) decreased significantly as dietary protein level increased: 13.2% for 30% CP versus 9.1% for 45% CP. This indicated that fish fed the lower protein diet were eating more feed as a percentage of body weight, possibly to compensate for the lower protein content of the diet. Additionally, a positive correlation was reported between weight gain and total protein intake (r^2 = 0.81).

Optimum energy-to-protein ratios have been determined for only a few species, but several studies indicate a ratio of 8 to 10 kcal of DE per g of CP in the diet is sufficient (Smith 1989). This value is similar to the values reported previously for pompano. Williams et al. (1985) proposed an optimum dietary DE:DP ratio for juvenile pompano of 7.4- 8.1 kcal DE/g DP, while Lazo et al. (1998) indicated adequate energy-to-protein ratios of 8.9-13.2 kcal/g diet. For the purpose of the present study, an intermediate digestible energy-to-crude protein (DE:CP) ratio of 9 kcal/g CP was chosen. A consistent DE:CP value across the diets would favor a suitable comparison among selected protein levels based on growth performance of juvenile Florida pompano.

Feed cost, particularly protein, is the major recurring cost in most aquaculture operations. It is therefore important to formulate species-specific diets in order to provide for the well-being of the animal as well as reduce unnecessary cost and waste. The objective of this study was to determine an optimum dietary protein level for Florida pompano fed diets containing a fixed digestible energy-to-crude protein (DE:CP) ratio, in an effort to formulate nutritionally complete, cost-effective diets for pompano.

Materials and Methods

Wild, juvenile pompano were collected, quarantined, and transported to the LSU ARS (see Appendix B), for acclimation to laboratory conditions in two identical marine recirculation systems. Each system consisted of twelve 200-L rectangular glass aquaria, a 1/3 HP pump (Sequence, MDM Incorporated, Colorado Springs, Colorado) for circulation, a bubble-washed bead filter (Aquaculture Systems Technologies, New Orleans, Louisiana), and an 80 watt UV sterilizer (Emperor Aquatics, Pottstown, Pennsylvania). A regenerative blower provided aeration to both systems via airstones in each tank. Each tank also had a coated wire mesh cover to prevent fish from jumping out, and a 50-W heater (Hydor, Sacramento, California) to maintain a constant water temperature. The outside glass of each aquarium was covered with black plastic to reduce stress on the fish caused by movements outside the tank. Fluorescent lighting was maintained for 24 hr because turning lights on and off excited the fish, causing them to hit the walls of the tanks.

During the acclimation period, fish were routinely checked for residual parasites and fed a commercial marine starter diet (50% CP, 14% lipid; Aquaxcel, Burris Mill and Feed, Franklinton, Louisiana). Prior to the start of the growth trial, a few randomly selected pompano underwent a disease screening at the LSU Aquatic Animal Disease Diagnostic Laboratory and were determined to be disease-free. Fish were hand-graded (mean initial weight 76 g/fish) and stocked at a uniform biomass and density of six fish per tank. Fish that died during the acclimation period were replaced by an individual of similar size. Three fish from the initial population were collected and frozen at -20^{o}C for analysis of body composition.

During the course of the growth trial, water quality (ammonia, nitrite, pH, alkalinity, DO, temperature, and salinity) was measured and the systems were maintained within ranges for warm-water, mesotrophic, fingerling growout (Malone and Pfeiffer 2006). Ammonia (0-0.2 mg/L) and nitrite (0-0.5 mg/L) were below the lethal concentrations reported for pompano by Weirich and Riche (2006). The pH of the systems was 7.6-8.2; alkalinity stayed above 100 mg/L; and DO was above 5 mg/L. Salinity was maintained at 10-12 ppt with synthetic sea salt (Crystal Sea, Marine Enterprises International, Baltimore, Maryland), and water temperature was maintained at 28^{o}C.

The experimental diets had six levels of protein (36%, 40%, 44%, 48%, 52%, and 56% CP). Diets were randomly assigned to two tanks of fish in each recirculation system, for a total of four replicates per treatment. The diets were computer-formulated (Mixit-Win, Agricultural Software Consultants, San Diego, California) with five primary ingredients (dehulled, solvent-extracted soybean meal; concentrated soybean protein; menhaden fish meal; menhaden fish oil; and a non-nutritive cellulosic filler), which were incorporated at graded levels (Table 3.1). The formulations utilized apparent digestibility values of gross energy determined in the aforementioned digestibility trials (Table 2.3), and were designed to provide a consistent DE:P ratio of 9 kcal DE/g CP. The diets were prepared in the manner described in Chapter 2. The proximate composition and gross energy content of the finished diets (Table 3.1) were determined by standard methods (AOAC 1995).

Fish were typically fed twice per day (morning and afternoon) to apparent satiation. Feed intake was monitored through a viewing window constructed in the black plastic covering the tank, which allowed observation of feed pellets on the tank bottom. Satiation was judged to occur when feeding activity slowed and few feed pellets remained on the tank bottom. The amount of diet that was offered at each feeding was recorded for each tank. Calculations used to assess the growth performance of the experimental diets are summarized in Table 3.2.

The growth trial lasted 12 weeks (87 d). During this time, the fish were anesthetized, weighed, and measured every three weeks. When fish were measured at stocking, they were individually marked with a Visible Implant Elastomer dye (VIE; Northwest Marine Technology, Shaw Island, Washington) injected into the clear tissue posterior to the eye (Figure 3.1). Earlier tests in our laboratory with this VIE marking system with pompano found the tag to have no effect on feeding or survival. The marking allowed the tracking of incremental growth of individual fish, as a supplement to average tank weights. Throughout the trial, dead fish were removed from the tank, identified, and weighed.

At the end of the growth trial, surviving fish were frozen at -20^{o}C for subsequent whole-body proximate analysis. Frozen carcasses were cut into pieces and freeze-dried for a minimum of 48 h (Lyphlock 18-L, Labconco, Kansas City, Missouri) to determine dry matter content. Dried whole-body samples were ground and homogenized with an electric coffee grinder, and stored at -20^{o}C until analyzed. Samples were pooled by tank, and analyzed in triplicate for proximate composition. Pooled samples were submitted to the LSU Agricultural Chemistry Department for determination of crude protein and crude fat content. Ash was determined by incineration in a muffle furnace at 600^{o}C, and

Table 3.1. Ingredient composition and proximate analysis of diets fed to Florida pompano, formulated to contain crude protein (CP) levels of 36% to 56% at a fixed digestible energy-to-protein ratio of 9.0 kcal/g CP. Values (as-fed) are expressed as percent of the diet unless otherwise noted.

	Treatments					
Ingredient	36-CP	40-CP	44-CP	48-CP	52-CP	56-CP
Dehulled, solvent extracted soybean meal (48% CP)	48.41	42.35	36.21	30.07	24.00	7.00
Concentrated soybean protein (85% CP)	2.80	4.60	6.40	8.20	10.00	20.00
Menhaden fish meal (62% CP)	12.30	20.50	28.75	37.00	45.19	51.23
Menhaden fish oil	10.82	11.68	12.53	13.38	14.23	16.73
Celufil (non-nutritive filler)	21.15	16.35	11.59	6.83	2.06	0.52
Calcium phosphate, monobasic	1.60	1.60	1.60	1.60	1.60	1.60
Soy lecithin, de-oiled	1.00	1.00	1.00	1.00	1.00	1.00
Carboxymethylcellulose	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin mix	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride (70%)	0.09	0.09	0.09	0.09	0.09	0.09
Vitamin C (Stay C 35)	0.06	0.06	0.06	0.06	0.06	0.06
Ethoxyquin (75%)	0.02	0.02	0.02	0.02	0.02	0.02
Total	*100.00*	*100.00*	*100.00*	*100.00*	*100.00*	*100.00*
Formulated to contain						
Digestible Energy (kcal/g diet)	3.24	3.60	3.96	4.32	4.68	5.04
Crude Protein	36.00	40.00	44.00	48.00	52.00	56.00
DE:P (kcal/g CP)	9.00	9.00	9.00	9.00	9.00	9.00
As analyzed						
Dry matter	93.6	93.3	93.2	93.1	93.4	92.8
Crude protein	34.6	39.0	42.1	47.0	50.8	54.5
Lipid	13.8	15.3	16.8	18.1	19.5	21.6
Fiber	9.7	8.9	8.0	5.3	4.2	2.9
Ash	7.4	8.8	9.6	11.0	12.4	12.6
N-free extract	28.1	21.3	16.6	11.7	6.5	1.2
GE (kcal/g diet)	4.7	4.8	4.9	5.0	5.1	5.2
GE:CP (kcal/g CP)	13.6	12.3	11.7	10.5	9.9	9.5

TABLE 3.2. Calculations used to assess the growth effects of six experimental diets, containing graded levels of protein (36% to 56% CP) and a fixed energy-to-protein ratio (9 kcal DE/g CP), on juvenile Florida pompano (mean initial weight 76 g). All calculations were applied to a tank of fish.

Variable		Formula
Survival (%)	=	(Final number of fish/Initial number of fish) x 100
Weight Gain (%)	=	[(g final wt. – g initial wt.) / g initial wt.] x 100
Average Biomass Gain (g)	=	Σ (individual wt. gain) / number of fish
Length Gain (%)	=	[(cm final length – cm initial length) / cm initial length] x 100
Specific Growth Rate (SGR, %)	=	[(ln (final wt.) – ln (initial wt.)) x 100] / number of days
Percent Feed Consumption (PFC, %)	=	(mean feed offered per feeding / mean body wt. of fish) x 100
Feed Conversion Ratio (FCR)	=	(g diet offered) / (g final biomass – g initial biomass + g dead fish)
Feed Conversion Efficiency (FCE, %)	=	(wet weight gain / dry feed fed) x 100
Fulton's Condition Factor (K)	=	(BW(g) x TL^{-3}(cm)) x 100
Protein Efficiency Ratio (PER)	=	weight gain (g) / protein fed (g; dry weight basis)

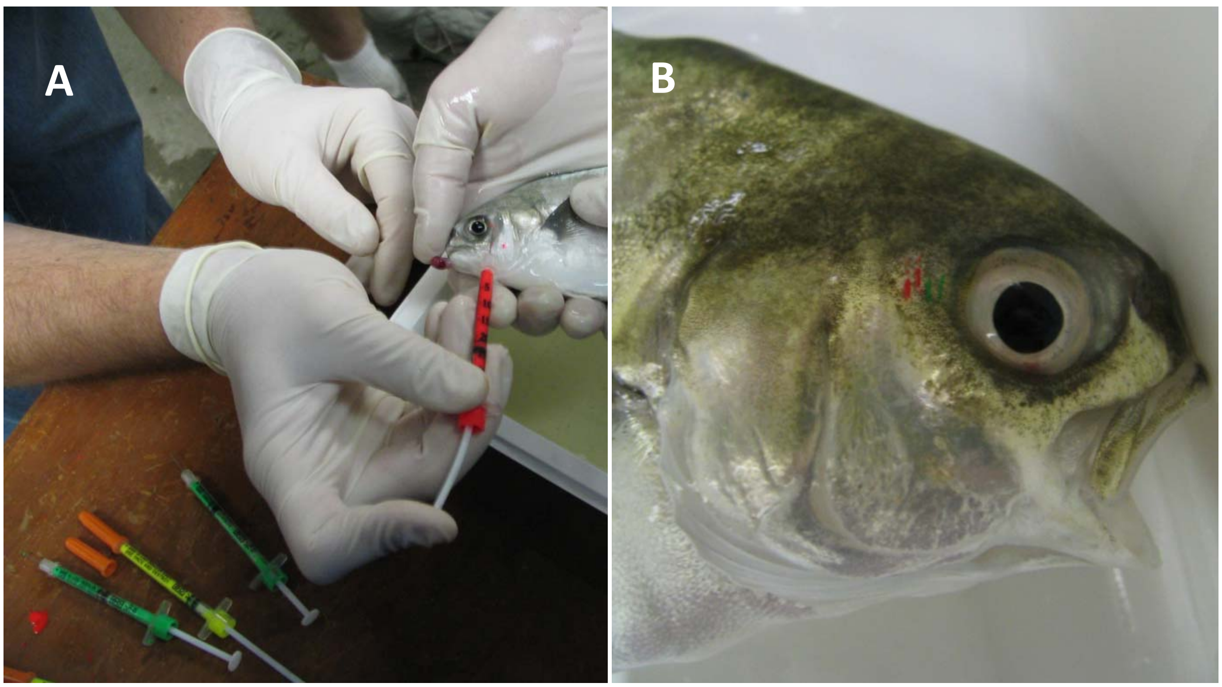

Figure 3.1. A) Injection of Visible Implant Elastomer (VIE) tagging system in juvenile Florida pompano, which was used to identify individual fish during the growth trial investigating effects of dietary crude protein levels. B) Use of multiple colors in the clear tissue posterior to the eye, on both sides of the fish, facilitated individual identification.

gross energy with adiabatic bomb calorimetry (Model 1241 adiabatic bomb calorimeter, Parr Instrument, Moline, Illinois).

Data were subjected to analysis of variance (ANOVA; SAS Institute 9.1, Cary, North Carolina) to determine the effects of dietary treatments on fish performance. Differences were considered statistically significant at $P \leq 0.05$. When differences were indicated, Tukey's Studentized range test was used to separate significantly different means. Broken-line regression was conducted (SAS 9.1) to determine the optimal dietary protein level (Robbins et al. 2006) for Florida pompano fed diets containing a fixed energy-to-protein ratio.

Results

Survival was lower than expected (0 to 67%), with only the 48-CP and 56-CP treatments finishing with greater than 50% mean survival. The 56-CP treatment had significantly higher survival than both the 36-CP and 40-CP treatments, while the 48-CP treatment had significantly higher survival than the 36-CP treatment (Figure 3.2.). Complete mortality was seen in one of the 44-CP treatment tanks and in all of the 36-CP treatment tanks, resulting in elimination of the 36-CP treatment at Day 45 (Figure 3.3). The majority of statistical analysis was therefore performed on data excluding the 36-CP treatment. Average weight gain ranged from 38 g to 89 g, and was significantly higher in both the 48-CP and 56-CP treatments compared to the 40-CP treatment (Table 3.3). Primary statistical analysis indicated a significant difference in percent weight gain; however, despite means ranging from 56% to 112%, Tukey's test of means found no significant differences among treatments (Figure 3.3, Table 3.3). Percent feed consumption (PFC) ranged from 3.0% to 3.8% per feeding, with 56-CP treatment significantly higher than the 40-CP treatment (Table 3.3). There were no significant differences in percent length gain or FCR (Table 3.3) among the protein levels. Although there were significant differences during portions of the growth trial, the final values of FCE (7 - 18%), PER (0.18 - 0.39), SGR (0.5 - 0.8%/d), and K (1.27 - 1.40) were not significantly different (Table 3.3 and 3.4).

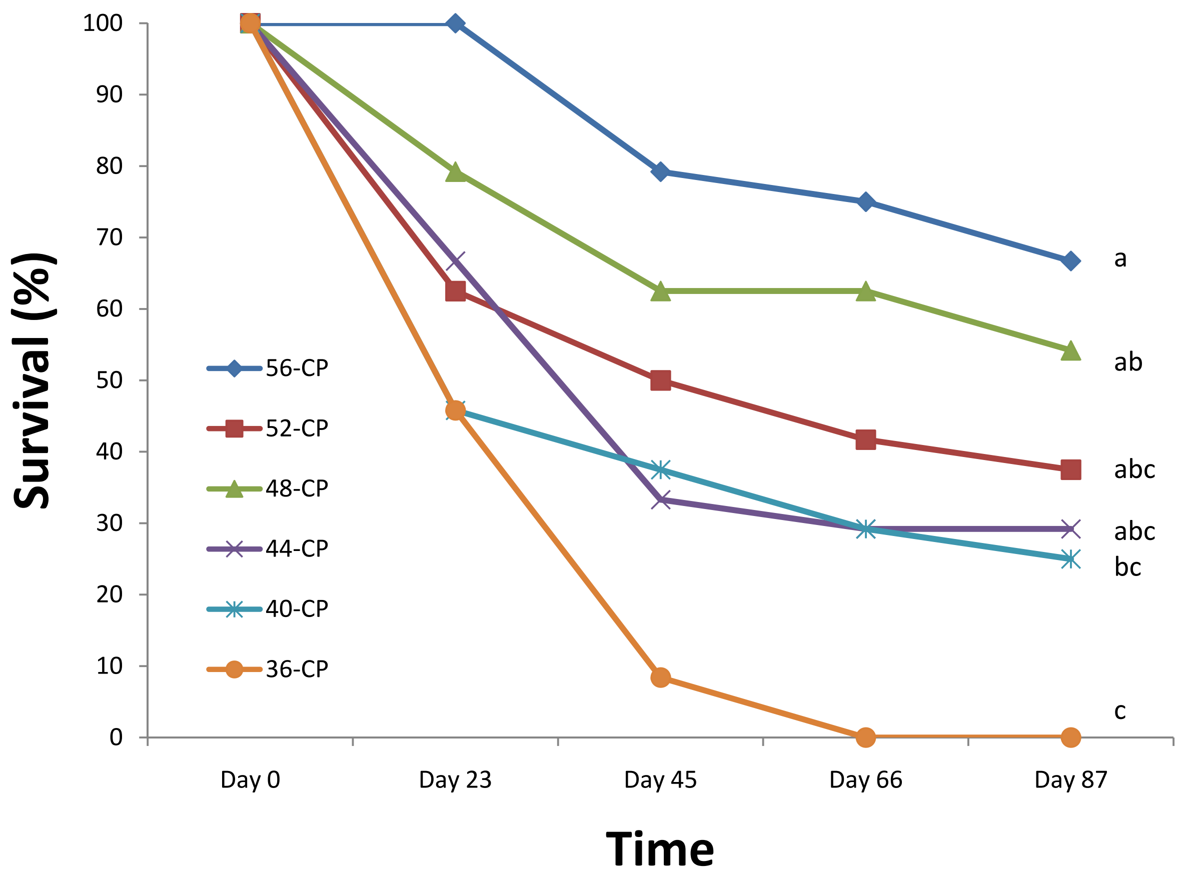

FIGURE 3.2. Survival (%) of juvenile Florida pompano fed diets with 36% to 56% crude protein (CP) at a fixed digestible energy-to-protein ratio of 9 kcal/g CP. Each point represents the mean of four tanks per treatment (six fish per tank). Final mean survivals with the same letter are not significantly different (P > 0.05).

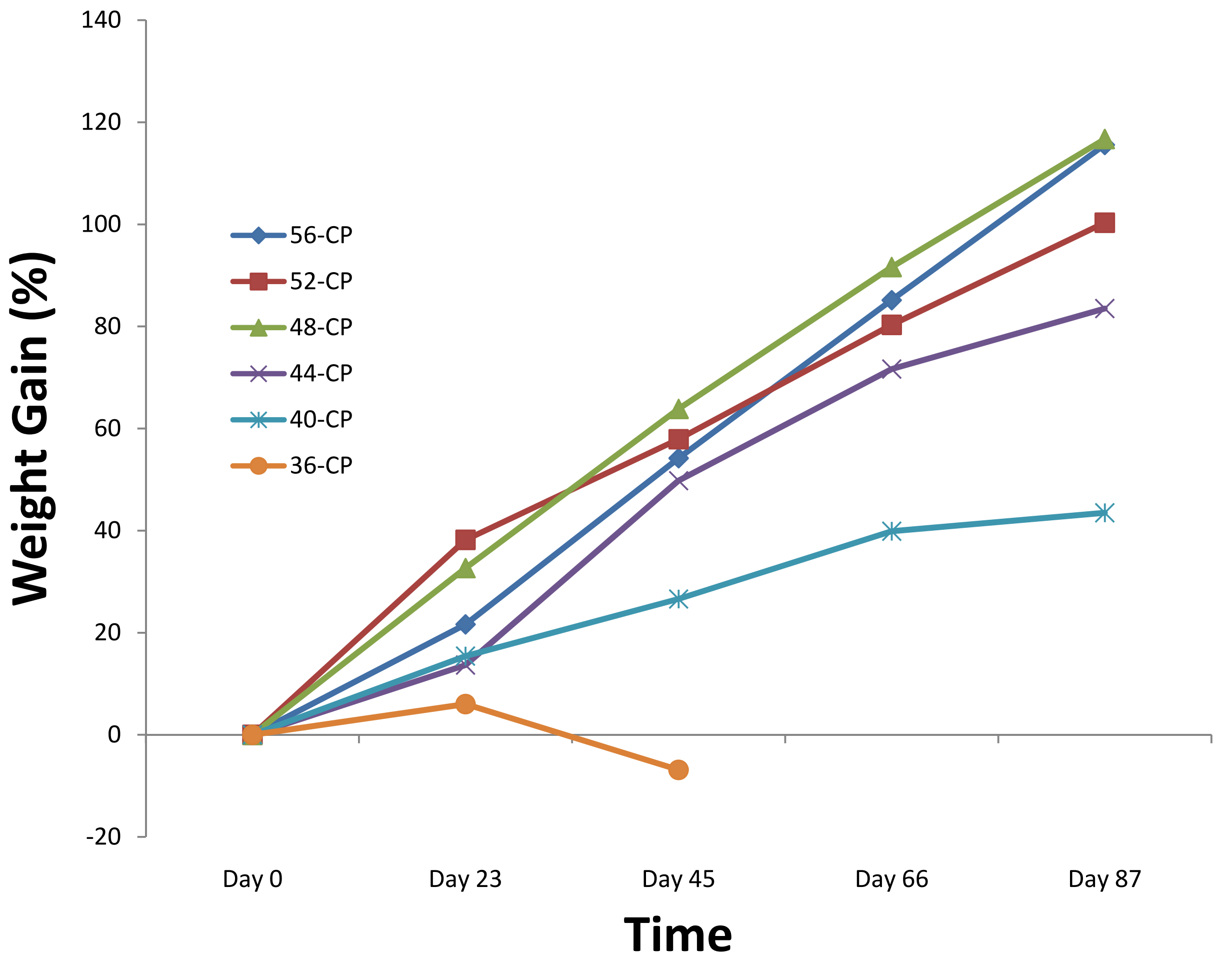

FIGURE 3.3. Weight gain (%) of juvenile Florida pompano fed diets with 36% to 56% crude protein (CP) at a fixed digestible energy-to-protein ratio of 9 kcal/g CP. Each point represents the mean of four tanks per treatment (mean initial weight 76 g). Poor survival resulted in elimination of the 36-CP treatment at day 45. Final weight gain (%) was not significantly different ($P > 0.05$).

Table 3.3. Final growth parameters calculated to assess the effects of dietary protein level in diets for juvenile Florida pompano (mean initial weight 76 g) containing a fixed energy-to-protein ratio (9 kcal DE/g CP) and fed for a period of 87 days. Within rows, values (mean ± SD) with the same letter are not significantly different ($P > 0.05$).

Parameter	Treatments				
	40-CP	**44-CP**	**48-CP**	**52-CP**	**56-CP**
Survival (%)	25.0 ± 9.6 b	29.2 ± 21.0 ab	54.2 ± 16.0 ab	37.5 ± 31.5 ab	66.7 ± 13.6 a
Weight Gain (%)	55.8 ± 10.6 a	82.3 ± 44.3 a	105.1 ± 16.5 a	75.6 ± 30.3 a	112.2 ± 20.6 a
Average Weight Gain (g)	38.3 ± 7.6 b	65.1 ± 39.4 ab	85.0 ± 7.7 a	59.9 ± 27.7 ab	89.1 ± 5.6 a
Length Gain (%)	14.0 ± 4.1 a	20.1 ± 12.1 a	25.6 ± 3.3 a	18.2 ± 5.2 a	26.7 ± 3.0 a
Specific Growth Rate (SGR, %/d)	0.5 ± 0.1 a	0.6 ± 0.3 a	0.8 ± 0.1 a	0.6 ± 0.2 a	0.8 ± 0.1 a
Percent Feed Consumption (PFC, %)	3.1 ± 0.2 ab	3.0 ± 0.3 b	3.4 ± 0.3 ab	3.3 ± 0.3 ab	3.8 ± 0.1 a
Feed Conversion Ratio (FCR)	12.4 ± 4.8 a	8.4 ± 6.7 a	5.5 ± 0.3 a	8.0 ± 4.0 a	5.5 ± 0.7 a
Feed Conversion Efficiency (FCE, %)	7.0 ± 5.8 a	12.8 ± 10.8 a	18.3 ± 0.9 a	15.0 ± 7.0 a	18.5 ± 2.2 a
Fulton's Condition Factor (K)	1.27 ± 0.07 a	1.38 ± 0.05 a	1.36 ± 0.05 a	1.38 ± 0.14 a	1.40 ± 0.04 a
Protein Efficiency Ratio (PER)	0.18 ± 0.15 a	0.31 ± 0.26 a	0.39 ± 0.02 a	0.30 ± 0.14 a	0.34 ± 0.04 a

TABLE 3.4. Incremental growth parameters calculated to assess the effects of dietary protein level in diets for juvenile Florida pompano (mean initial weight 76 g) containing a fixed energy-to-protein ratio (9 kcal DE/g CP) and fed for a period of 87 days. Within columns, values (mean ± SD) for variables with the same letter are not significantly different (*P* > 0.05).

		Days				
Variable	Treatment	0 - 23	24 - 45	46 - 66	67 - 87	0 - 87
Weight (g)	40-CP	85.8 ± 2.9 a	94.3 ± 11.7 a	104.1 ± 9.8 a	106.8 ± 11.4 a	
	44-Cp	84.2 ± 25.2 a	116.7 ± 31.7 a	133.8 ± 41.8 a	143.1 ± 48.5 a	
	48-CP	103.0 ± 14.5 a	126.8 ± 12.4 a	148.1 ± 12.5 a	167.4 ± 21.6 a	
	52-CP	101.5 ± 17.4 a	116.8 ± 37.3 a	133.4 ± 40.8 a	148.8 ± 51.9 a	
	56-CP	96.7 ± 7.7 a	122.5 ± 12.8 a	147.2 ± 13.6 a	171.4 ± 16.4 a	
Weight gain (%)	40-CP	11.9 ± 11.1 a	9.0 ± 3.9 a	7.3 ± 7.0 a	1.8 ± 6.7 b	30.0 ± 19.8 b
	44-Cp	10.8 ± 10.0 a	15.9 ± 6.5 a	15.1 ± 4.6 a	6.1 ± 3.2 ab	38.6 ± 31.4 ab
	48-CP	25.8 ± 2.3 a	18.0 ± 4.9 a	16.5 ± 3.3 a	8.1 ± 4.3 ab	68.4 ± 12.9 ab
	52-CP	22.9 ± 9.8 a	14.5 ± 10.3 a	8.6 ± 9.8 a	8.0 ± 6.9 ab	54.1 ± 21.1 ab
	56-CP	21.7 ± 4.3 a	19.4 ± 8.0 a	17.0 ± 4.0 a	15.7 ± 2.6 a	73.7 ± 13.4 a
FCE (%)	40-CP	10.0 ± 9.3 b	4.9 ± 2.9 a	7.6 ± 9.8 a	2.4 ± 8.2 a	7.0 ± 5.8 a
	44-Cp	13.6 ± 13.4 b	18.4 ± 14.2 a	19.7 ± 11.8 a	7.5 ± 6.0 a	12.8 ± 10.8 a
	48-CP	37.3 ± 9.9 a	15.5 ± 7.0 a	20.4 ± 1.5 a	7.8 ± 4.1 a	18.3 ± 0.9 a
	52-CP	23.0 ± 6.0 ab	16.0 ± 14.3 a	21.1 ± 27.8 a	-2.3 ± 17.7 a	15.0 ± 7.0 a
	56-CP	22.9 ± 2.1 ab	18.8 ± 4.5 a	18.9 ± 2.9 a	14.9 ± 2.9 a	18.5 ± 2.2 a
PER	40-CP	0.26 ± 0.24 b	0.13 ± 0.07 a	0.20 ± 0.25 a	0.06 ± 0.21 a	0.18 ± 0.15 a
	44-Cp	0.32 ± 0.32 b	0.44 ± 0.34 a	0.47 ± 0.28 a	0.18 ± 0.14 a	0.31 ± 0.26 a
	48-CP	0.79 ± 0.21 a	0.33 ± 0.15 a	0.43 ± 0.03 a	0.17 ± 0.09 a	0.39 ± 0.02 a
	52-CP	0.45 ± 0.12 ab	0.31 ± 0.28 a	0.41 ± 0.54 a	-0.05 ± 0.35 a	0.30 ± 0.14 a
	56-CP	0.42 ± 0.04 ab	0.35 ± 0.08 a	0.35 ± 0.05 a	0.28 ± 0.05 a	0.34 ± 0.04 a
SGR (%/d)	40-CP	0.43 ± 0.43 a	0.37 ± 0.18 a	0.32 ± 0.31 a	0.07 ± 0.31 b	0.50 ± 0.08 a
	44-Cp	0.40 ± 0.39 a	0.66 ± 0.27 a	0.66 ± 0.19 a	0.27 ± 0.14 ab	0.65 ± 0.30 a
	48-CP	0.95 ± 0.05 a	0.75 ± 0.19 a	0.72 ± 0.14 a	0.36 ± 0.19 ab	0.81 ± 0.09 a
	52-CP	0.85 ± 0.35 a	0.59 ± 0.41 a	0.37 ± 0.44 a	0.36 ± 0.31 ab	0.63 ± 0.21 a
	56-CP	0.80 ± 0.14 a	0.79 ± 0.31 a	0.74 ± 0.15 a	0.69 ± 0.11 a	0.85 ± 0.10 a
K	40-CP	1.21 ± 0.03 b	1.26 ± 0.05 b	1.31 ± 0.13 a	1.33 ± 0.12 a	1.27 ± 0.07 a
	44-Cp	1.23 ± 0.04 ab	1.32 ± 0.08 ab	1.44 ± 0.07 a	1.42 ± 0.06 a	1.38 ± 0.05 a
	48-CP	1.29 ± 0.05 ab	1.42 ± 0.02 a	1.42 ± 0.03 a	1.43 ± 0.05 a	1.36 ± 0.05 a
	52-CP	1.26 ± 0.07 ab	1.39 ± 0.10 ab	1.38 ± 0.11 a	1.39 ± 0.13 a	1.38 ± 0.14 a
	56-CP	1.34 ± 0.07 a	1.41 ± 0.01 a	1.40 ± 0.07 a	1.41 ± 0.04 a	1.40 ± 0.04 a

No significant differences in whole-body composition were observed between treatment groups and pre-trial fish; however differences were detected among treatment groups (Table 3.5). Dry matter (DM) ranged from 27% to 31%, with 56-CP having significantly higher DM than 40-CP. The remaining proximate analysis results are expressed on a dry weight basis. Crude protein (CP) ranged from 53% to 60% and lipid content ranged from 25% to 32%, neither had significant differences. Ash content ranged from 9% to 12%, with 40-CP having significantly higher levels than 56-CP.

Broken-line regression was performed on the percent weight gain data, which indicated that 46.3% CP was the optimum dietary protein level for juvenile Florida pompano fed a diet containing 9 kcal of DE/g of CP, under the conditions of this study (Figure 3.4).

Discussion

The effects of the six dietary treatments on fish weight gain and feed efficiency indicated that the diet with 48% CP and a DE:P ratio of 9 kcal/g CP was optimum for juvenile Florida pompano under the conditions of this study. This CP level provided the greatest number of positive results among the measured parameters, at the lowest level of dietary protein. However, the use of broken-line regression identified a growth plateau at an optimum (and untested) protein level of 46.3% CP. This dietary protein level would be more economical than 48% CP, and it agrees favorably with the previously reported optimal protein level for juvenile pompano of 45% (Lazo et al. 1998). This value is also within the range of 40% to 55% reported for a variety of carnivorous fish species (NRC 1993, Wilson 2002).

The low survival seen in the study was unexpected, and the exact reasons for it are unknown. The 36-CP diet was poorly consumed by pompano, with fish in this treatment either refusing to eat or exhibiting very little feeding activity. Over half of the fish in this treatment died during the first 3 weeks of the growth trial, and the remainder lost weight. A possible reason for the poor feed response could be a palatability issue, as was seen with red drum, another marine carnivorous species fed diets with soybean meal as the sole source of protein (Ellis and Reigh 1991). The poor palatability of diets for

TABLE 3.5. Whole body proximate composition of pre-trial and surviving Florida pompano fed diets with 40% to 56% crude protein and a digestible energy-to-protein ratio of 9 kcal/g CP. Values are expressed on a dry-matter basis. Values for pre-growth trial pompano are included for comparison purposes, not statistical analysis. In each column, values with the same letter are not significantly different ($P > 0.05$).

Diet	Dry Matter (%)				Protein (%)				Lipid (%)				Ash (%)			
pre-trial fish	28.3	±	3.5		58.4	±	0.9		26.3	±	0.9		11.7	±	0.5	
40-CP	27.2	±	1.5	b	59.6	±	5.9	a	25.1	±	4.9	a	11.9	±	0.9	a
44-CP	28.9	±	0.8	ab	57.8	±	1.0	a	28.2	±	0.5	a	10.7	±	1.2	ab
48-CP	30.2	±	0.4	ab	53.1	±	1.4	a	31.3	±	2.2	a	10.1	±	1.0	ab
52-CP	28.1	±	2.7	ab	59.4	±	4.9	a	25.0	±	3.8	a	10.4	±	1.3	ab
56-CP	30.6	±	0.6	a	54.3	±	2.5	a	32.3	±	2.2	a	9.2	±	0.2	b

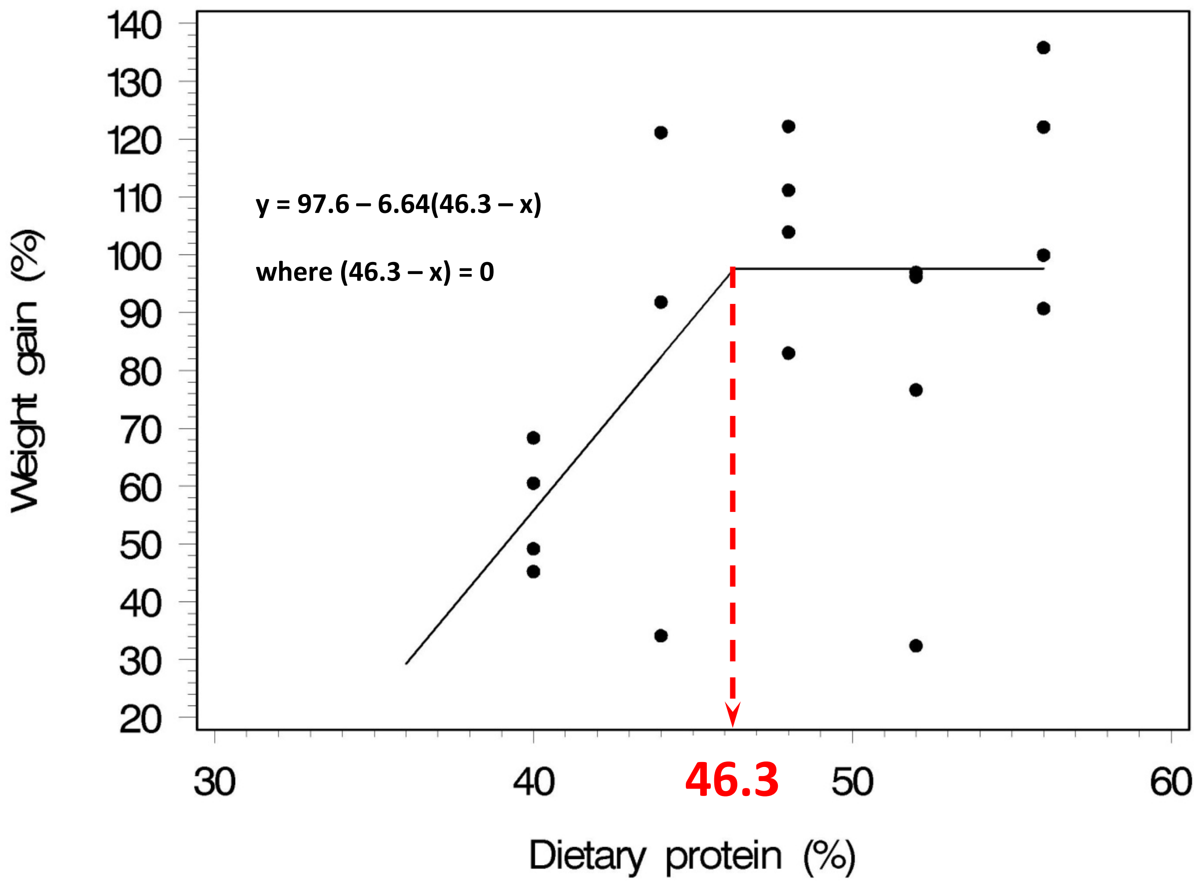

FIGURE 3.4. Cumulative weight gain (%) of pompano from surviving tanks after 87 days. One-slope broken-line regression model determined the optimal dietary protein level for pompano to be 46.3% for diets with 9 kcal of digestible energy per g of crude protein.

red drum containing high levels of soybean meal was overcome with the inclusion of 10% CP from fish meal in a later study (McGoogan and Gatlin 1997). The 36-CP diet had high levels of carbohydrates (fiber + N-free extract = 44%) principally from soybean meal (48% of diet) and cellulose (21% of diet); however, the presence of 12% fish meal and 11% fish oil was not sufficient to stimulate consumption of this diet.

Poor survival of the remainder of these wild-caught juvenile pompano may have been related to stress and culture conditions. The more nutrient-complete, higher-protein diets did have a positive effect on survival. An exception to this trend was the low survival in three of the four replicates for the 52-CP treatment (16.7% survival in two replicates and 33.3% in one replicate) compared to the 48-CP and 56-CP treatments (33-67% and 50-83% survival, respectively). Unfortunately the low survival had an impact on data analysis by increasing the variation in results and elimination of some experimental replicates. Exacerbating the problem was non-uniform fish sizes, because death of a large fish had a greater impact on average size measurements than death of a small fish. The use of the VIE dye allowed for growth measurements on individual fish in each tank, thus some growth parameters could be measured.

Despite poor survival, when measurements of individual weight gain were used to determine average weight gain by tanks significantly greater fish growth was observed in treatments with higher protein levels. It cannot be determined if differences in weight gain obtained in this study and those reported in the literature are significant, due to differences in culture conditions, but meaningful comparisons can be made. Values obtained for percent weight gain and SGR in this study are much lower than values previously reported for juvenile pompano (Table 3.6). Individuals from multiple treatments in the current study achieved impressive weight gain in 87 days (44-CP = 143 g, 48-CP = 180 g, 52-CP = 137 g, 56-CP = 125 g); although, other fish from the same tanks grew poorly or lost weight thus negatively impacting average growth parameters. Variability in growth rates of pompano has been

Table 3.6. Dietary composition and growth parameters [initial weight (g), final weight (g), days of growth trial, specific growth rate (SGR), percent weight gain] for six growth trials with juvenile Florida pompano. A description of each study's objective is listed below the associated citation. The "Trt/Results" column represents the treatment for the lowest (L) and highest (H) values obtained for each study.

Study	Diet		Trt/Result	Initial Wt. (g)	Final Wt. (g)	Days	SGR (%/d)	Wt. Gain %
	CP %	Lipid %						
Current Study CP levels	*40*	*12*	*40% - L*	*76*	*106.8*	*87*	*0.5*	*56*
	56	*17*	*56% - H*	*76*	*171.4*	*87*	*0.8*	*112*
Lazo et al. 1998 CP levels	30	8	30% - L	4.5	23.4	49	3.4	420
	45	8	45% - H	4.5	30.7	49	3.9	582
Williams et al. 1985 Fish oil levels	42	0	oil-free	1	2.17	48	1.6	117
	42	12	12% - L	1	4.3	48	3.0	330
	42	8	8% - H	1	5.1	48	3.4	410
Weirich et al. 2006 Fixed feeding frequency	56	14	1x/d – L	17	56.3	38	3.1	231
	56	14	3x/d – H	17	64.5	38	3.6	279
Weirich et al. 2006 Fixed vs satiation w/density	56	14	Fixed/LD - L	73.9	181	54	1.6	145
	56	14	Sat./LD - H	73.9	213.6	54	1.9	189
Weirich et al. 2006 Satiation feeding frequency	56	14	2x/d – L	218.5	691.4	133	0.9	216
	56	14	4x/d - H	218.5	753.9	133	0.9	245

reported (McMaster 1988, Weirich et al. 2006), and remains a potential obstacle to successful pompano culture. Because wild caught fish were used in this study, the differential growth exhibited here may be overcome by selective breeding for desirable growth characteristics.

While not significantly different, the trends exhibited for the final values of percent length gain (LG%), Fulton's condition factor (K), specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE), and protein efficiency ratio (PER) indicate that pompano utilized higher protein diets more effectively. The increasing values for LG% indicate that weight gain included muscle growth, and not just fat deposition. Increases in both length and weight provide the basis for the K values (1.27 to 1.40), which indicate the fish in the study to be in good condition. The FCR and FCE values (5.5-12.4 and 7-19%, respectively) for pompano in this study, were poorer than previously published studies with pompano (FCR = 1.9-4.0, Williams et al. 1985; FCR= 3.1-6.6, Gomez and Scelzo 1982; FCR = 1.4-3.0, Heilman and Spieler 1999; FCE = 30%, Tatum 1972; FCE = 31-51%, Lazo et al. 1998), which could be a result of poor survival. Furthermore, the lower conversion efficiencies of pompano in this study, compared to other species of marine fish, could be a product of the high metabolic demand of this extremely active fish. Lack of significant differences in PER indicate that the energy provided in the diets was adequate at the protein levels tested.

It has been suggested that fishes, like most animals, eat to satisfy energy needs (Smith 1989). Consequently, growth depression can occur if energy intake suppresses appetite before sufficient protein has been consumed to support maximum growth (Ellis and Reigh 1991). Using diets containing 42% CP, Williams et al. (1985) suggested that the high energy diet (12% fish oil) might have limited feed consumption in pompano and consequently produced lower weight gain, protein gain, and feed conversion compared to diets containing 4% and 8% fish oil. This study indicates that diets for pompano containing a fixed dietary protein level have a corresponding optimum dietary energy level; however, changes in dietary protein content also impact feed consumption. In a study using isoenergetic diets, Lazo et al. (1998) reported pompano fed a diet with 45% CP and 8% lipid (4% fish oil) had significantly lower average percent daily feed consumption (PDFC) than fish fed a diet with 30% CP and 8% lipid (4.75% fish oil). The fish fed the low protein diet consumed more feed (presumably to compensate for the lower protein content of the diet), which corresponds to more total energy consumed. These results imply pompano growth may not be limited by energy content alone; instead growth can be maximized with an optimum ratio of energy-to-protein in the diet (DE:DP = 8.1; GE:P = 8.9, from Williams et al. (1985) and Lazo et al. (1998), respectively).

In the current study using a fixed energy-to-protein ratio, pompano fed the diet containing the highest protein and lipid level (56% CP and 16.7% fish oil; 54.5% CP and 21.6% lipid, as analyzed) had significantly higher average percent feed consumption (PFC) than fish fed the diet containing 40% CP and 11.7% fish oil (39% CP and 15.3% lipid, as analyzed). The PFC values, which represent the average amount of diet offered per feeding (twice per day) indicate that juvenile pompano fed the 56-CP diet actually consumed more, on a per unit weight basis, than fish fed the 40-CP diet despite the higher energy and lipid content. A potential cause for the increased consumption of the high protein diet in the current study may be related to the high FM content compared to the lower protein diet which contained a higher level of SM. However, all PDFC values for this study (6.0-7.6%) were lower than PDFC values in the Lazo et al. (1998) study (9.1-13.2%) which used smaller fish (Table 3.6). Additionally, Weirich et al. (2006) found that juvenile pompano fed a commercially prepared diet containing 56.5% CP and 13% lipid did not self-limit feed consumption regardless of the total energy consumed. They consistently found pompano to consume more feed, thus grow more, when provided the opportunity (apparent satiation [AS] > fixed rate; AS four times per day > AS twice per day) despite the high energy content of the diets. High levels of feed consumption resulted in higher whole-body lipid levels (48%) of pompano compared to results from the current study (25.0-32.3%) and other pompano studies (13.9-22.6%, Williams et al. 1985; 18.5-21.6%, Lazo et al. 1998).

In order to ensure essential nutrients are not limiting, diets are often formulated to be over-fortified especially with regard to protein levels. Unfortunately, if nutrients are not balanced, excess protein is metabolized and either excreted into the environment or converted to fat. Until the specific amino acid requirements of a species are identified, gross “requirements” for protein can be used as a guide for diet formulation. Dietary protein requirements may vary due to a number of factors including fish size, water temperature, protein quality, feed allowance, and non-protein energy (NRC 1993). The results of this study indicate the optimum dietary protein level for juvenile pompano is approximately 46.3% for diets containing 9 kcal DE/g CP, under the culture conditions reported here. This value is comparable to that reported from other studies with Florida pompano and can be useful in the formulation of nutritionally complete, least-cost diets for pompano until more research is performed.

CHAPTER 4
SUMMARY

Improved growth with increased dietary protein levels, as observed in this growth study, are typical of pompano and other carnivorous species. However economic viability of pompano production depends on formulating a least-cost diet that provides the nutrients required to maximize growth. Based on the results of this research, a diet containing 46% CP and 9 kcal DE/g CP was optimum for these culture conditions. In order to formulate a diet containing these nutrient levels, ingredients of known composition must be used. Chemical composition of these ingredients is only partially helpful, because the quality of an ingredient hinges on the availability of the nutrients present. Thus research involving quantification of feedstuff digestibility and nutrient availability is equally important as determinations of gross nutrient requirements for the overall goal of formulating nutritionally complete diets for Florida pompano.

Protein is the most important, and expensive, component of aquaculture diets. Too little protein in the diet results in reduced growth, while too much (or an imbalance) results in only a portion used to make new protein with the remainder converted to energy. As previously discussed, protein quality is a product of the amino acid profile and the nutrient availability of the ingredient. Thus it is imperative to consider both aspects when choosing feedstuffs for diet formulation. Although the results reported in this digestibility study provide detailed information on the nutrient availability of four common feedstuffs, proper least-cost diet formulation requires a much larger selection of ingredients from which to choose. As the nutrient availability of additional dietary protein sources is determined for pompano, diets can be formulated to meet the nutrient requirements to achieve rapid growth, while simultaneously reducing the amount of fish meal in diets for this carnivorous marine fish.

Additional research should be done on dietary energy-to-protein ratios for pompano. Results of the current study indicate that the DE:P ratio of 9 kcal/g CP was sufficient for rapid growth of pompano;

however, it may not necessarily be the optimum ratio. Weirich et al. (2006) concluded that energy levels in the commercial diet they used (13% lipid) appeared to be too high because of high levels of whole-body lipid in the pompano at harvest. The lipid levels of pompano were lower in this study than the aforementioned study, but still higher than other pompano nutrition studies (Williams et al. 1985, Lazo et al. 1998). The pompano in this study consumed less feed per day (6.0-7.6% bw/d) compared to another study (9.1-13.2% bw/d) in which CP levels were investigated (Lazo et al. 1998). This difference could be related to the use of larger fish in the current study thus comparatively less feed (% bw/d) was needed to achieve satiation. Regardless, total energy content of the diet did not reduce feed intake, with the fish receiving the highest energy content consuming the most diet. The reduced feed consumption observed in the lower energy diets may be the result of poor palatability of soybean meal and the non-nutritive cellulose-based filler (Celufil), especially compared to the voracious feeding observed with the diets that contained comparatively higher levels of fish meal.

Furthermore, practical diets should be investigated that utilize multiple energy sources (besides fish oil). Williams et al. (1985) suggested that the optimum level of fish oil in diets for pompano is 4-8% based on increased weight gain and protein gain compared to diets containing 12% fish oil. The values used in this study were higher (11-17%); however, rapid growth of pompano was reported by Weirich et al. (2006) with diets containing 13% lipid. Lipids are highly available energy sources for fish, while utilization of carbohydrates is highly variable (NRC 1993). Both are less expensive energy sources than protein, so care should be taken to utilize proper amounts of digestible energy from non-protein feedstuffs in order to spare protein for growth. The optimum dietary energy levels for this highly active fish need to be determined at different dietary protein levels.

A wide range of growth variation existed among individual fish at the end of the growth trial in this study, which has been reported in previous studies (Berry and Iverson 1967, Weirich et al. 2006). While a high degree of size variation might be expected among fish fed restricted rations, fish in the current study and the Weirich et al. (2006) study were fed to apparent satiation. Apparent satiation feeding should allow all fish to consume adequate amounts of feed for similar growth rates, but that was not the case in either of these studies, both of which used wild-caught juvenile pompano. A possible cause for the variable growth could be sexual dimorphism, whereby females grow larger than males of the same age; however this study was concluded before fish reached sizes at which they become sexual mature. If the variable growth was not a product of sex-selection, but instead simply variable growth among individuals, then this type of rapid growth could be selectively bred into future generations of cultured pompano.

Spawning techniques have been refined for pompano, and subsequent pompano nutrition studies in our laboratory have utilized fish produced by researchers (Harbor Branch Oceanographic Institution [HBOI], Ft. Pierce, Florida) and by commercial producers (Dyer Aqua, Sebastian, Florida). Use of cultured juveniles reduces the risk of contamination from naturally occurring parasites and diseases inherent with wild-caught juveniles. While the details of commercial pompano production are largely proprietary, publications have been produced by HBOI involving broodstock care and conditioning, spawning, and larval rearing of pompano (Weirich and Riley 2007). Most of the problems associated with marine aquaculture production involve larval rearing, so continued research is needed to refine techniques to produce more juveniles. Another aspect of current research in other laboratories is investigation of low-salinity production of pompano. Studies have shown that pompano can tolerate salinities near 0 ppt; however, it has yet to be seen if growth rates can be maximized at extremely low salinities. Results from this type of research would have significant impacts on production costs for inshore facilities like the LSU ARS which must rely on manufactured sea salt.

The consistently high market value of Florida pompano could provide producers with a potentially high profit margin. Feed quality will affect the rate of growth and cost of growing pompano to market size. The diets used in this growth trial were semi-practical diets formulated with a limited set of ingredients in order to evaluate the growth effects of graded protein levels in combination with a fixed energy-to-protein ratio. Recent research with a commercially available diet (53% CP, 13% lipid) has indicated the feasibility of rearing pompano from 17 g to market size (450-700 g) in 4-8 months (Weirich et al. 2006). Nutritional research can reduce the feed cost associated with pompano production by reducing over-fortification of diets through development of species specific formulations. Data reported in this study can facilitate the formulation of nutritionally complete, cost-effective diets for Florida pompano.

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APPENDIX A

ABBREVIATIONS USED IN THIS THESIS

AA	amino acid
AAAA	apparent amino acid availability
ACD	apparent carbohydrate digestibility
ACFD	apparent crude fat digestibility
ACPD	apparent crude protein digestibility
ADC	apparent digestibility coefficient
AEAAA	apparent essential amino acid availability
AED	apparent energy digestibility
ARS	Aquaculture Research Station
AS	apparent satiation
bw	body weight
^{o}C	degrees Celcius
CG	corn grain
cm	centimeter
CP	crude protein
CPS	corrected protein score
d	day
DE	digestible energy
DO	dissolved oxygen
DP	digestible protein
EAA	essential amino acid
FCE	feed conversion efficiency
FCR	feed conversion ratio
FD	freeze-dried
FM	fish meal
g	gram
GE	gross energy
GI	gastrointestinal
HBOI	Harbor Branch Oceanographic Institution
HP	horsepower
HPLC	high pressure liquid chromatography
hr	hour
I/B	intestine length/body length
ICP	inductively coupled plasma spectrometry
K	Fulton's condition factor
kcal	kilocalorie
kg	kilogram
L	liter
LG	length gain
LSU	Louisiana State University
LUMCON	Louisiana Universities Marine Consortium
m	meter
MB	meat and bone meal
mg	milligram
min	minute
mm	millimeter
N	nitrogen
NMFS	National Marine Fisheries Service
NOAA	National Oceanic and Atmospheric Administration
OD	oven-dried
PDFC	percent daily feed consumption
PER	protein efficiency ratio
PFC	percent feed consumption
ppm	parts per million
ppt	parts per thousand
PS	protein score
SD	standard deviation
SGR	specific growth rate
SM	soybean meal
TL	total length
UV	ultraviolet
VIE	Visible Implant Elastomer
W	watt
WBP	whole body pompano

Appendix B

Collection and quarantine of juvenile Florida pompano

Wild-caught Florida pompano (mean weight 2 g) were collected during the summer with a 6-m long, 0.6-cm mesh, minnow seine in the surf zone of Grand Isle, Louisiana. The collection procedure consisted of multiple semi-circular pulls with the net, using the beach as a barrier, in order to capture juvenile fish swept to shore by the wave action. Captured pompano were temporarily placed in a 19-L bucket filled with water, then moved to a live-hauler filled with water from the surf zone, and aerated with compressed oxygen. Ice was introduced to lower water temperature thereby reducing handling and transport stress on the fish. An ammonia-binding agent (Ammo-lock, Mars Fishcare, Chalfont, Pennsylvania) and a stress reducer (Stress-Coat, Mars Fishcare, Chalfont, Pennsylvania) were also added to the hauler water.

The juvenile pompano were transported to the Louisiana Universities Marine Consortium (LUMCON) in Cocodrie, Louisiana, where they were quarantined for at least two months to remove naturally occurring parasites. Upon arrival at LUMCON, the fish underwent a gradual acclimation to freshwater and received a freshwater-formalin dip (150 ppm) for a period of one hour in order to remove gill parasites such as *Amyloodinium* spp. and *Paratrichodina* spp. They were then placed in a recirculation system containing 0.25 mg/L chelated copper (Cutrine-Plus, Applied Biochemists, Germantown, Wisconsin) for additional gill parasite treatment. The fish were also treated with 2.5 mg/L praziquantel (Ecological Laboratories, Cape Coral, Florida) to destroy monogenetic trematodes. During the quarantine period, pompano were acclimated to a commercial marine starter diet (50% CP, 14% lipid; Aquaxcel, Burris Mill and Feed, Franklinton, Louisiana) via automatic feeders. After quarantine, the fish were transported to the Louisiana State University Agricultural Center's Aquaculture Research Station (ARS) in Baton Rouge, Louisiana, where they were acclimated to laboratory conditions.

VITA

Craig Thomas Gothreaux was born in November of 1980 in Scott, Louisiana. In 1988 his family moved to Baton Rouge, Louisiana, where he eventually enrolled at Louisiana State University in 1998 with the help of the TOPS Scholarship. The first two years of college were spent in architecture, after which he transferred into the School of Renewable Natural Resources (RNR). While in RNR, he was the recipient of the Rockefeller Scholarship, the John H. “Red” Lehmann Scholarship, and the Forestry, Wildlife and Fisheries Alumni Scholarship. In the summer of 2002, he ran a business (Acadian Lobster and Seafood) that specialized in importing lobsters from Nova Scotia, holding them at a lobster-holding facility in Scott, and distributing them to restaurants around the south Louisiana area. At the same time, he undertook a self-assigned research project under Dr. Robert C. Reigh investigating the growth rate of American lobsters fed a commercial shrimp diet held at two different temperatures. While the experiment and the lobster business did not pan out, it did introduce him to the field of aquaculture. The next school year he started working at the LSU Aquaculture Research Station for Dr. Reigh as an undergraduate student worker until graduating with his Bachelor of Science degree in wildlife conservation in December of 2003. Ineffably fascinated by fish, he decided to shift his focus for graduate school. In January of 2003 he started his graduate program under Dr. Reigh researching nutritional information on Florida pompano. Over his college career, he has been an active member of the LSU and Baton Rouge Rugby Clubs, World Aquaculture Society, and the Louisiana Chapter of the American Fisheries Society (LAAFS), as well as a member of the Golden Key International Honor Society, Gamma Sigma Delta Honor Society, and the National Society of Collegiate Scholars. He has presented his research at a number of professional society meetings, and has received the award for Best Student Oral Presentation (LAAFS – 2006) and Best Student Poster Presentation (LAAFS – 2008). He is currently a candidate for the Master of Science degree in fisheries, with a specialization in aquaculture.